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Biochemical studies on the effect of *Ecballium elaterium* "fruit juice as an antioxidant against hepatotoxicity induced by paracetamol

M. Sc. – Dissertation By

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DEDICATION TO

MY FAMILY

AND

MY FRIENDS

First and foremost I wish to give all the praise to almighty ***ALLAH*** for giving me strength and time to complete this thesis.

I thank my family, *my father*, *mother*, *brothers* and *sisters,* also offer my heartfelt gratitude to my best friend ***Abdullah Allawaj*** for great support and encouragement in my study.

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LIST OF ABBREVIATIONS

ABBREVIATION	The word
Abs	Absorbance.
ALP	Alkaline phosphatase.
ALT	Alanine aminotransferase.
AMP	Adenosine monophosphate.
ASC^{2-}	Ionized ascorbate.
$Asc^{\bullet -}$	Semidehydroascorbate radical.
$ASCH^{-}$	Mono-acid ascorbate.
$AscH^{\bullet}$	Tricarbonyl ascorbate free radical.
$ASCH_2$	Di-acid ascorbate.
AST	Aspartate aminotransferase.
ATP	Adenosine Tri phosphate.
ATPase	Adenosine Tri phosphatase.
BCG	Bromcresol green.
BRN	Bilirubin.
b.w.	Body weight.
°C	Degree Celsius.
CAT	Catalase.
CGC	Cucurbitacin glucose combination.
CoQ-10	Coenzyme Q.
Cuc	Cucurbitacin.
Cu-Zn SOD	Copper-zinc SOD.
CYP	Cytochrome P450.

CYP2A6	Cytochrome P450 2A6.
CYP2E1	Cytochrome P450 2E1.
d.f.	Degree of freedom.
dl	Deciliter.
DNA	Deoxyribonucleic acid.
$DPPH^{\cdot}$	1,1-diphenyl-2-picrylhydrazyl.
E. elaterium	*Ecballium elaterium.*
ET	Electron transfer.
FAD	Flavin adenine dinucleotide.
Fe (II)	Ferrous.
Fe (III)	Ferric.
$Fecl_3$	Ferric Kchloride.
Fig.	Figure.
Figs.	Figures.
FMN	Flavin mononucleotide.
g	gram.
G-GT	Gama glutamyl transferase.
GOT	Glutamate oxaloacetate transferase.
GPT	Glutamate pyruvate transferase.
GPx	Glutathione peroxidase.
GR	Glutathione reductase.
GSH	Reducing glutathione.
GS-SG	Oxidizing glutathione.
GST	Glutathione *S*-transferase.

GST Pi	Glutathione *S*-transferase Pi.
h	Hour.
HAT	Hydrogen atom transfer.
HClO	Hypochlorous acid.
$HNO_2^{\bullet}$	Nitrous acid.
H_2O_2	hydrogen peroxide.
$HOO^{\bullet}$	Hydroperoxyl radical.
HPLC	High performance liquid chromatography.
kDa	Kilo Dalton.
kg	Kilogram.
l	Liter.
LC-ESI-MS	liquid chromatography-electrospray ionization-mass spectrometry
LD_{50}	Moderate lethal dose.
LDH	Lactate dehydrogenase.
LDL	Low density lipoprotein.
LPO	Lipid peroxidation.
M	Molar.
MDA	Malondialdehyde.
MDH	Malate dehydrogenase.
MFO	Mixed function oxidase.
mg	Milligram.
min.	Minute.
ml	Milliliter
mM	Milimolar.

Mn-SOD	Manganese-SOD.
Mo	Molybdenum.
MPT	Mitochondrial permeability transport.
MPO	Myeloperoxidase.
MRSA	Methicillin resistant Staphylococcus aureus.
n	Number of measurements.
NACLAR	National Advisory Committee For Laboratory Animal Research.
Na_2CO_3	Sodium carbonate.
NAD^+	Nicotine amide adenine dinucleotide, oxidized.
NADH	Nicotine amide adenine dinucleotide, reduced.
NADPH	Nicotine amide adenine dinucleotide phosphate, reduced.
NAPQA	*N*-acetyl-p-benzoquinone imine.
NAPSQI	N-acetyl-p-benzosemiquinone imine.
N.B.	Nota bene.
nm	Nanometer.
No.	Number.
NO	Nitric oxide.
$NO^{\bullet}$	Nitric oxide radical.
$NO_2^{\bullet}$	Nitrogen dioxide radical.
NOS	Nitric oxide synthase.
4-NPP	4-Nitrophenyl phosphate.
ns	Nano second.
O_2	Oxygen.
$O_2^{\bullet}$	Superoxide anion.

$^{\bullet}OH$	Hydroxyl radical.
8-OHdG	8-hydroxy-2-deoxyguanosine.
$ONOO^{-}$	Peroxynitrite anion.
ONOOH	Peroxynitrous acid.
$ONOOCO_2^{-}$	Nitrosoperoxycarbonate.
P	Probability.
PCM	Paracetamol.
PCM-gluc	Paracetamol glucuronide.
PCM-GS	Paracetamol glutathione.
PCM-Slup	Paracetamol sluphate.
PhOH	Phenolic antioxidants.
PMNs	Polymorphonuclear neutrophils.
PSMO	Polysubstrate mono oxygenase.
PUFA	Polyunsaturated fatty acid.
Redox	Reduction oxidation reaction.
R-NH2	Amines.
RNH-Cl	Chloramines.
RNS	Reactive nitrogen species.
$ROO^{\bullet}$	Peroxyl radical.
ROOH	Hydroperoxide.
ROS	Reactive oxygen species.
RPA	Reducing power assay.
rpm	Round per minute.
RSA	Radical scavenging activity.

S	Serum.
S.D.	Standard deviation.
S.E.	Standard error.
SGOT	Serum glutamate oxaloacetate transferase.
SGPT	Serum glutamate pyruvate transferase.
SOD	Superoxide dismutase.
TBA	Thiobarbituric acid.
TFC	Total flavonoid content.
TNF-α	Tumor necrosis factor-alpha.
TPC	Total phenolic content.
U/L	Unite per liter.
VLDL	Very low density lipoprotein.
X	Sum of the individual values.
$(\bar{X})$	Arithmetic mean.
Σd^2	Sum of squared deviations of the values of the variant from the arithmetic mean of series.

ABSTRACT

The study was designed to investigate the antioxidant and hepatoprotective effects of *Ecballium elaterium* "Fruit juice". The phytochemical analysis of *E. elaterium* "Fruit juice" indicates the presence of Phenols and flavonoids. Total Phenolics content and flavonoids were found to be 132.46 µg/ml of pyrogallol and 26 µg/ml of quercetin which equivalent to 100 µl of *E. elaterium* "fruit juice" respectively. The antioxidant activity of *E. elaterium*" fruit juice" ranged from 37% at 100µl to 89% at 500 µl. Where found each 100 µl of fruit juice equivalent to 30 µg/ml of vitamin C. The reducing capacity of the *E. elaterium*" fruit juice" found to be 230.17 µg/ml of ascorbic acid equivalent to 100 µl of *E. elaterium* "fruit juice".

The hepatotoxicity induced by paracetamol at 400 mg/kg body weight in male albino rats every 48 h for 21 days. The protective effect of *E. elaterium* against paracetamol induced liver toxicity in male rats was monitored by liver marker enzymes levels. Level of marker enzymes such as "ALT, AST, ALP, G-GT and total bilirubin" significantly increased, also MDA level increased, but the level of total protein was decreased in addition to antioxidant enzymes "GR, GPx, CAT, SOD" in paracetamol treated rats. The fruit juice of *E. elaterium* at 100 µl induced hepatoprotective effect by decreasing the increase of liver marker enzymes, these effects were noted in prophylactic and curative groups the activity of *E. elaterium* "fruit juice" 100 µl where compared with vitamin C at 300 mg/kg body weight in prophylactic group, in addition with vitamin C at 300 mg/kg and 50 mg/kg body weight of silymarin in curative group.

The histopathological studies also supported the protective properties of *E. elaterium* "fruit juice". The area of necrosis and degeneration of hepatocytes were observed in the toxic group. The prophylactic and curative groups showed a marked protective effect with decreased necrotic zones and hepatocellular degeneration.

Key word: *Ecballium elaterium*, phytochemical, pyrogallol, hepatotoxicity.

INTRODUCTION

The liver is sometimes referred to as the "great chemical factory" of the body, because the body depends on the liver to regulate, synthesize, store and secrete many important proteins, nutrients, chemical and to purify and clear toxin substances from the body. Most importantly the liver is considered to be the center of metabolic transformation of drugs and other toxins. Liver damage is always associated with cellular necrosis, increase in lipid peroxidation and depletion in the glutathione levels. In addition serum levels of many biochemical markers like serum of AST, ALT, ALP and bilirubin are elevated **[1]**.

Hepatotoxicity is one of the very common aliment resulting into serious debilities ranging from severe metabolic disorders to even mortality. Hepatotoxicity in most cases is due to free radical and oxidative damage caused by free radicals may be related to aging and related diseases **[2,3]**. This has been implicated in the pathogenecity of major diseases like atherosclerosis, cirrhosis, diabetes and cancer **[4]**.

Paracetamol is commonly used analgesic and antipyretic drug. The paracetamol is safe at therapeutic levels, but an acute paracetamol overdose can lead to potentially fatal hepatic necrosis in humans and experimental animals. It has been found that reactive oxygen species (ROS) and reactive nitrogen species (RNS) take an important part in the development of hepatotoxicity which caused by paracetamol.

The initial step of its toxicity is cytochrome P450 metabolism of paracetamol to the reactive intermediate N-Acetyl-P-benzoquinone imine (NAPQI). At therapeutic dose this metabolite is removed by conjugation with glutathione (GSH). However at large doses of paracetamol conjugation with GSH leads to its depletion **[5]**.

In the absence of a reliable liver protective drugs and because undesirable side effects of synthetic agents, there is growing focus to follow systematic research to evaluate on scientific basis the traditional herbal medicines that are claimed to possess hepatoprotective activity **[2]**. Antioxidant compounds reduce the action of reactive oxygen species (ROS) in damaged tissues during the recovery process **[6]**.

It is logical to consider antioxidants as primary candidates to counteract such toxic effect. Accumulating evidence supported the protective effects of antioxidants from medicinal plants against oxidative stress mediated disorders. Studies are going throughout the world for the search of protective molecules that would provide maximum protection of the liver, kidney as well as other organs very little or no side effects **[5]**.

Fruits and vegetables are the major sources of dietary antioxidant vitamins, such as vitamin C, vitamin E and phenolic compounds which are also antioxidant and are numerous and widely distributed in the plant kingdom**[7]**. Phenolic compounds are widely distributed in the plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging, anti-inflammatory and anti carcinogenic **[8]**. Phenolic constituents, such as flavonoids and phenolic acids are especially worthy of notice due to their high antioxidative activity **[9]**.

The natural antioxidants are known to play an important role in protection against disorders which caused by oxidant damage **[10]**.

Antioxidants refer to compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions, they act in one or more of the following ways: as reducing agents, by free radical scavenging, and as quenchers of reactive oxygen species (ROS) **[11]**.

AIM OF THE WORK

The few studies conducted on *Ecballium elaterium*, encouraged us to study the antioxidant and hepatoprotective effects of this plant to enrich knowledge on the biological activities of this plant. For these reasons, we are interested in studying the antioxidant activity of *Ecballium elaterium* fruit juice in addition to prophylactic and curative effect against hepatotoxicity in albino rats.

REVIEW OF LITERATURE

Plant products are widely used in folk medicine because of their low toxicity and great medicinal value. Much research have been concentrated on different plant extracts abilities to induce antioxidant effects **[12].**

Plants continue to be a major source of medicine, as they have always been used throughout human history. These plants have been reported to exert therapeutic efficacies through their antioxidant activities. Today, medicinal plants are showing tremendous promise for preventive intervention in the pathogenesis of many diseases, as well as in their treatment **[13].**

The usage of herbal drugs for the treatment of diseases has been increased all over the world. The herbal drugs are believed to be harmless and free from serious adverse reactions as they are obtained from nature and are produced through biological systems. Also the limited therapeutic options and disappointing therapeutic success of modern medicine has increased the usage of alternative medicine including herbal preparations **[14].** Plants used in traditional medicine have shown to be effective against various disorders with free radical involvement. Some plants being used in traditional medicine also show wonderful properties against free radical mediated disorders.

However, the exact chemistry of these plants is not fully established. Even in case where chemical constituents are established, the correlation between activity and constituent is not clearly verified. Pharmacological evaluation of such plant could help us understand their activity, mechanism of action and potential for therapeutic use **[15].**

The chemical content of polyphenolic compounds such as flavonoids and related polyphenols also vitamins such as C, E, in plants were considered to be the active components in natural product according to its activity as an antioxidant compounds and in treatment of diseases. One of the known plants that used in the folk medicine is *Ecballium elaterium* "Fruit juice".

1. *Ecballium elaterium*:

1.1. General:

Ecballium elaterium (L) (squirting cucumber or donkey's green) is a perennial plant from the family of Cucurbitaceae; *Ecballium elaterium* has a large fleshy root. The leaves are petiolate, large, and rough. The flowers are yellow and axillary. The fruit has the shape of a small oval cucumber, about an inch and a half long and one inch thick. It has a greenish or grayish color, and is covered with stiff hairs or prickles. When fully ripe, it separates from the peduncle, and throws out its juice and seeds with considerable force through an opening at the base, where it was attached to the footstalk. The name "squirting cucumber" comes from the tendency of the ripe fruit to explode upon the slightest touch, with its dark seeds and juice ejected at a distance of several meters. The ripe fruit is about 4 cm long of yellow-green color **[16].**

Ecballium elaterium (Cucurbitaceae) is a wild Mediterranean medicinal plant which has been described to thrive in drastic environmental conditions. In one study, it has been described to be frost-tolerant as compared to other wild species of the Cucurbitaceae family **[17].** *Ecballium elaterium* is interest today because its fruits extracts are still used in Mediterranean region in different medicinal system. The diluted aqueous extract of *Ecballium elaterium* fruits is a traditional anti-inflammatory and analgesic for chronic sinusitis. It also possesses other uses especially the treatment of fever, cancer and liver disorders **[18].**

1.2. Phytochemistry of *Ecballium elaterium*:

Knowledge of the chemical constituents of plants is desirable, not for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substance. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies **[19].** The fruit juice of *Ecballium elaterium* contains proteins, lipids, sugars, and minerals which support its nutritional value of this plant **[20].**

1.2.1. Phenolic compounds:

Phenolic compounds are a large group of phytochemicals widespread in the plant kingdom. Depending on their structure they can be classified into simple phenols, phenolic acids, hydroxycinnamic acid derivatives and flavonoids. Phenolic compounds have received considerable attention for being potentially protective factors against cancer and heart diseases, in part because of their potent antioxidative properties and their ubiquity in a wide range of commonly consumed foods of plant origin. Phenolic compounds is a generic term that refers to a large number of compounds widely dispersed throughout the plant kingdom and characterized by having at least one aromatic ring with one or more hydroxyl groups attached **[21].**

The constituent of *Ecballium elaterium* contains phenolics as minor components include hydroquinol, 2-nitroquinol, 4-hydroxy acetophenone, 4-hydroxy-3-methoxy acetophenone **[22].**

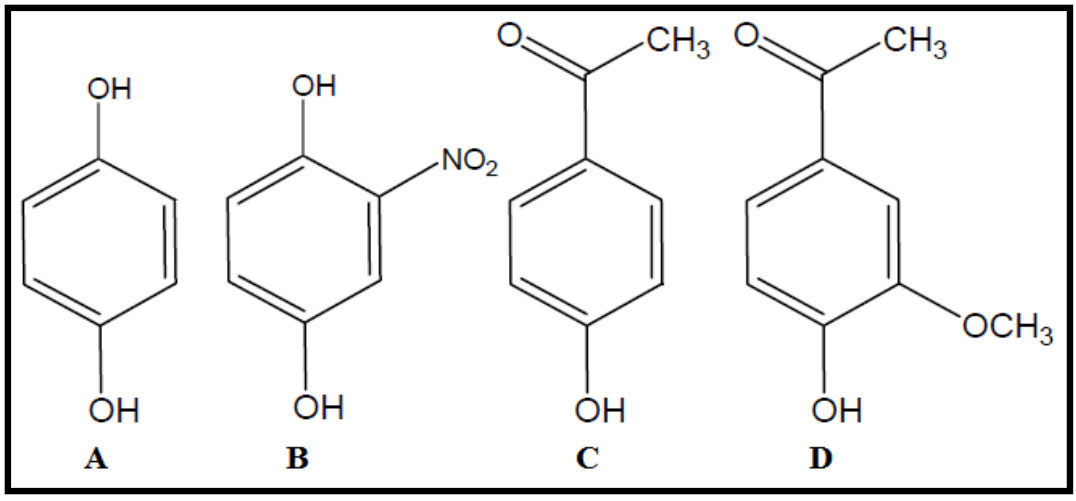


Fig.(1): A: hydroquinol, B: 2-nitroquinol, C: 4-hydroxy acetophenone, D: 4-hydroxy-3-methoxy acetophenone.

Recently, growing interests on phenolic compounds focused on their biological activities linking to human health benefits, such as antioxidant. The polyphenols are the most important phytochemicals, because they possess many biological activities and health-promoting benefits **[23].**

Phenolic compounds have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals. The antioxidative potential of phenolic compounds can be attributed to their strong capability to transfer electrons to free radicals, chelate metal ions, activate antioxidant enzymes and inhibit oxidases.

Phenolic compounds often play a primary or a synergistic function. They are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also their ability to form stable radical intermediates, which prevent various food ingredients from oxidation **[24].** These phenolic compounds may work by providing hydrogen atoms from their phenolic hydroxyl groups to scavenge hydroxyl radical generated from hydrogen peroxide and thus protect leukocytic DNA from damage induced by H_2O_2 **[25].**

1.2.2. Flavonoids:

Flavonoids are polyphenolic compounds comprising fifteen carbons with two aromatic rings connected by a three-carbon bridge. They are the most numerous of the phenolics and are found in most members of the plant kingdom. They are present in high concentrations in the epidermis of leaves and fruits and have important and varied roles as secondary metabolites, being involved in processes like, pigmentation, stimulation of nitrogen-fixing nodules, disease resistance and protect the plant from u.v. rays of the sun **[21].**

The antioxidant ability of flavonoids and phenolic acids is related to the number and position of hydroxyl groups in the molecule; an increase in the number of hydroxyl groups leads to a higher antioxidant activity. Compounds with three hydroxyl groups on the phenyl ring of phenolic acids or the B ring of flavonoids have a high antioxidant activity **[26].**

Ecballium elaterium is rich source of phytomelin, also known as rutin, quercetin-3-o-rutinoside with antioxidant effect. The amount of Phytomelin per 1.0 g of dry powder of flowers = 1.59 ± 0.12 mg, fruits = 1.84± 0.13 mg, and leaves = 8.54 ± 0.56 mg **[27].**

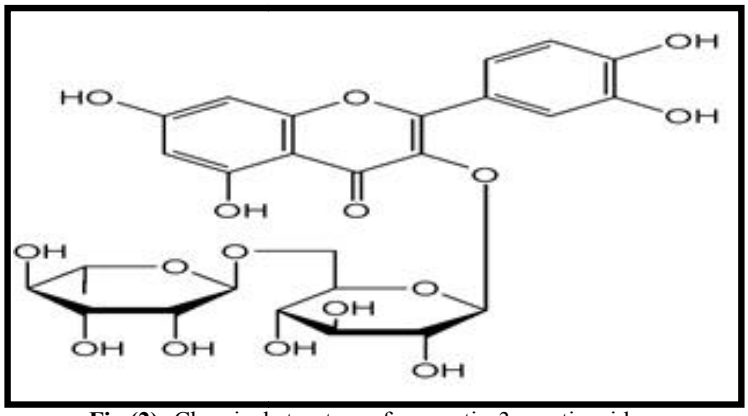


Fig.(2): Chemical structure of quercetin-3-o-rutinoside.

Flavonoids of plant origin are reported to have potent antioxidants and homeostatic balance between pro-oxidant and anti-oxidants they are known to be important for maintenance of health as well as prevention from various degenerative diseases. Flavanoids constitute major group of compounds which act as primary antioxidants and are known to react with hydroxyl radicals, superoxide anion radicals, and lipid peroxyradicals, protect DNA from oxidative damage, inhibitory against tumor cell and possess anti-inflammatory and antimicrobial properties **[28].**

Since the phenolic compounds in dietary sources exhibit potent free radical-scavenging properties, their main role was thought to be as antioxidants involved in protection against lipid peroxidation. it is necessary to know the bioavailability of polyphenols and their metabolites, to evaluate their biological activity in target tissues **[29].** Furthermore, epidemiologic studies suggest a protective role of dietary flavonoids against coronary heart disease. The association between flavonoid intake and the long term effects on mortality was studied subsequently and it was suggested that flavonoid intake is inversely correlated with mortality due to coronary heart disease. An important effect of flavonoids is the scavenging of oxygen derived free radicals. In vitro experimental systems it was also showed that flavonoids possess anti-inflammatory, antiviral, and anticarcinogenic properties **[30]**.

Flavonoids can inhibit LDL oxidation in vitro this action protects the LDL particles and may have preventive action against atherosclerosis. When flavonoids are

used as antioxidants, free radicals are scavenged and therefore can no longer react with nitric oxide, resulting in less damage.

Interestingly, nitric oxide can be viewed as a radical itself, and it was reported that nitric oxide molecules are directly scavenged by flavonoids. Therefore, it has been speculated that nitric oxide scavenging plays a role in the therapeutic effects of flavonoids **[30].**

1.2.3. Cucurbitacins:

The natural cucurbitacins constitute a group of triterpenoid substances. Structurally they are characterized by the tetracyclic cucurbitane skeleton **[31].** The building block of terpenes is isoprene (2-methyl 1,3-butadiene) precursor of essential oils found in many plants **[16].** The primary cucurbitacins formed in *Cucurbitaceae* plants are cucurbitacin B and E. Other cucurbitacins could have been produced from the primary cucurbitacins **[32].**The cucurbitacin E, B, D and I have been found in all plant tissues analyzed (root, stem, leaf, flower, and fruit) of *Ecballium elaterium*. Appreciable amount were found in the fruit but only trace amounts in the other tissues **[33].**

Until now a large number of cucurbitacin derivatives have been isolated from the fruit juice. Including two cucurbitacin glycosides which have been isolated and characterized from the fruit juice, their aglycones were cucurbitacin B and cucurbitacin D, while the sugar moiety in both cases was glucose **[34].**

Ecballium elaterium, a medicinal plant, whose fruit juice is used for the treatment of jaundice in folk medicine, has been reported as being capable of decreasing bilirubinemia in animals with jaundice **[35].** In Turkey, the fresh fruit juice of this plant is directly applied into the nostrils for the treatment of sinusitis as a herbal folk remedy **[27].** Ethanolic extract of *Ecballium elaterium* fruits is very efficient in treating infectious diseases caused by methicillin resistant *Staphylococcus aureus* MRSA and may also helpful for treating diseases caused by *Candida albicans*.

However, to explain the mode of action, the active phytocompounds of this plant used against multidrug-resistant bacteria and their toxicity, they have to be determined by additional studies **[18].**

The juice of *Ecballium elaterium* is fiber free but contains proteins, lipids, sugars, and minerals. The extract of the juice, analyzed by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS), contains cucurbitacins (Cuc) B fig. (3A), as well as several glycosylated compounds **[20].**

The antioxidant properties of cucurbitacin B glucosides fig. (3B) and cucurbitacin E glucosides fig. (3C) (cucurbitacin glucoside combination, CGC) and their direct free radical scavenging properties were established **[37].**

Antioxidant activity was measured by the ability of the CGC to inhibit MDA formation during the oxidation of linoleic acid. The inhibition of MDA formation demonstrates that the CGC exhibits antioxidant properties, probably through the involvement of a direct scavenging effect on several free radicals **[37].**

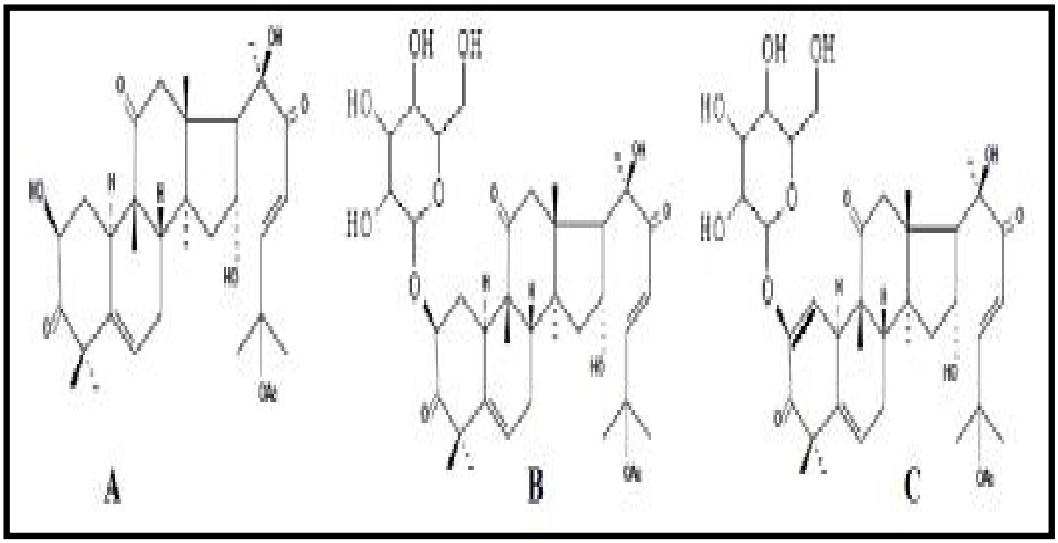


Fig.(3): A: Cucurbitacin B, **B:** Cucurbitacin B glycoside, **C:** Cucurbitacin E glycoside

2. Paracetamol (Acetaminophen)

2.1. Chemical structure:

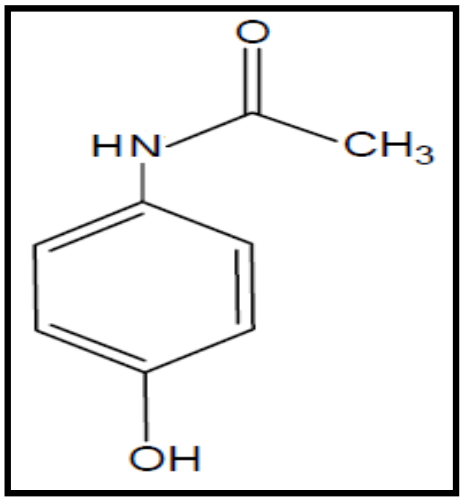


Fig. (4): Chemical structure of Acetaminophen

2.2. General:

Acetaminophen (Paracetamol, PCM, and N-acetyl-p-aminophenol) was first discovered in 1893. Since then, it has become one of the most commonly used analgesic and antipyretic drugs in the treatment of mild to moderate pain. It is considered one of the safest minor analgesics when taken at the recommended therapeutic dose. Although it possesses similar antipyretic and analgesic properties as aspirin (acetylsalicylic acid), it does not have significant anti-inflammatory activity. However, many people still consider it more desirable than aspirin because, unlike aspirin, it does not cause gastrointestinal ulceration **[37].**

In overdose, it produces centrilobular hepatic necrosis in humans and animals. It is well established that the formation of the reactive metabolite *N*-acetyl-p-benzoquinone imine (NAPQI) during the metabolism of acetaminophen by cytochrome P_{450} is an important step in the development of the hepatotoxicity **[38].**

2.3. Biotransformation of Acetaminophen:

Many lipophilic xenobiotic compounds enter the body and are deposited in adipose depots and other tissues. Several classes of these chemicals are known to contribute to the risk of cancer and death. Accidental exposure to high level of some lipophilic xenobiotic results in chronic disease affecting the liver **[39].** Drug biotransformation is usually done through an enzymatic process which occurs in two phases.

- **Phase I:** metabolism which involves oxidation, reduction and hydrolysis of the foreign compound. The primary function of this stage is to introduce polar groups (e.g. alcohols) into the parent compound thereby providing sites for conjugation in Phase II reactions.
- **Phase II:** reactions which include glucuronidation, sulfation, GSH and amino acid conjugation. These enzymes use the exposed polar group to covalently link an endogenous molecule to create a conjugate. The polysubstrate monooxygenase system (PSMO; mixed function oxygenase; cytochrome P450 system) represents a large number of Phase I enzymes involved in biotransformation **[40].**

These enzymes insert 1 atom of oxygen into their substrates and this action requires the presence of cofactor NADPH (sometimes NADH) and the oxygenated form of the cytochrome P450 (CYP), P450 is a membrane bound enzymes located primarily in the smooth endoplasmic reticulum. Many Phase II enzymes are soluble and are found in the cytosol of cells. While both Phase I and Phase II reactions have generally been thought to generate non toxic products, research in the last two decades has emphasized that they may actually bioactivate some compounds to be more toxic metabolites **[37].**

The liver has been found to be the primary target of xenobiotic-induced toxicity for several reasons:

1. The liver receives and reacts with all the blood, containing many nutrients and foreign chemicals, which come from the gastrointestinal tract via the portal vein, as well as blood from the systemic circulation.

2. The enzymes *(e.g.* CYP_{450}) that catalyze the biotransformation reactions are located in high concentrations in the liver.

3. The liver has the capacity to extract and chemically modify many different compounds from the blood prior to storing, secreting into the bile or releasing them into the general circulation, making it more likely to be affected by toxic compounds **[40].**

Hepatic drug metabolizing enzyme system is called mixed function oxidase or monooxygenase containing many enzymes including phase I enzymes such as CYP where play a prominent role in the metabolism of many pharmaceutical agents and activation or deactivation of potential carcinogens. Acquiring metabolic information and determining the effect of chemicals on hepatic drug metabolizing enzymes are important in developing clinically safe and efficient medications **[41].**

The mechanism involved in liver injury is related to the fact that small amounts of acetaminophen are converted to a toxic metabolite. The toxic metabolite binds with liver proteins to cause cellular injury. The amount of toxic metabolite produced and the ability of the liver to remove this metabolite before it binds to liver protein influence the extent of liver injury **[42].**

Acetaminophen (paracetamol) one of the most widely used analgesic drugs, is safe at therapeutic doses, but causes liver failure in overdoses **[43].** When a normal dose is used paracetamol is metabolized primarily by glucuronidation and sulfation. These major conjugates paracetamol sulfate (PCM-sulp) and paracetamol glucuronide (PCM-gluc), being more water soluble than the parent compound, are eliminated from the liver and blood mainly via urine and a little via bile (PCM-gluc). About 30% to 55% of administered paracetamol is excreted in urine as (PCM-sulp) and (PCM-gluc) respectively **[44].** Normally, toxic oxidation metabolites generated in the liver are converted into non-toxic metabolites excreted in urine via conjugation with glutathione (GSH) containing sulphydryl groups **[41].**

A small amount of acetaminophen is probably metabolized via a third metabolic pathway, that is, oxidation by the microsomal cytochrome P450 (CYP) containing mixed function oxidase system (MFO) to NAPQI. Its adducts to glutathione, cysteine

conjugate and mercapturic acid breakdown products were found in human urine after ingestion of acetaminophen **[45].** Exposure to high doses of acetaminophen increases the generation of (NAPQI) a highly electrophilic metabolite that triggers liver damage **[41].** Although a minor oxidation reaction, hydroxylation of acetaminophen to 3-hydroxy acetaminophen is probably also occurring in human as methylated 3-hydroxy-acetaminophen has been found in urine of patients who had taken an overdose of acetaminophen **[46].**

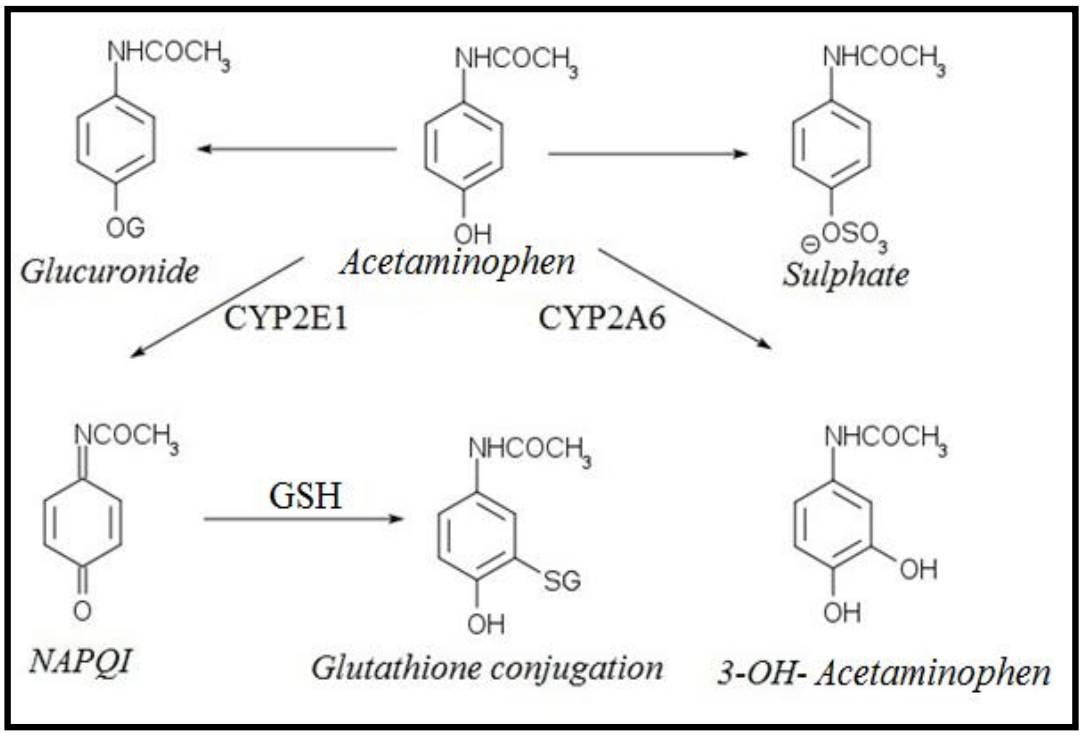


Fig. (5): metabolism of acetaminophen (paracetamol)

2.4. Mechanisms of hepatotoxicity:

2.4.1. General:

The hepatotoxicity of acetaminophen occurs mainly with the formation of excess NAPQI via cytochrome P_{450}. Hepatotoxic damage occurs mainly in the centrilobular zone. As the formation of NAPQI is regarded to be the rate-limiting step in the formation of the acetaminophen glutathione (PCM-SG) conjugate, the high concentration of cytochrome P_{450} enzymes in the perivenous region is probably more important for the observed zonal toxicity than the low GSH content **[47].**

The initial biochemical reactions between the reactive metabolites and macromolecular cell components (proteins, lipids, DNA), were grouped and called Stage I, whereas subsequent processes of adaptation or failure of response to modification of essential cellular processes (such as energy supply and the protein machinery) were grouped in Stage II of toxicity **[48].**

Several mechanisms could contribute in acetaminophen toxicity "hepatic damage"

The first hypothesis is that covalent protein binding of NAPQI as an electrophile is the most important event, leading to disrupted homeostases once critical proteins have been modified **[49].** The oxidative stress, i.e. thiol oxidation, mediated by the oxidative capacities of NAPQI, is the main cause of hepatotoxicity. NAPQI can oxidize GSH, thereby lowering the GSH/GSSG ratio, and it can oxidize SH groups in protein, leading to the formation of interstrand disulfide bridges, to interprotein cross linking, or to mixed disulfides between protein and glutathione.

The second hypothesis is that oxidative stress accompanied often by lipid peroxidation (LPO) as caused by a redox cycling metabolite of acetaminophen is the crucial step **[50].** Covalent binding of NAPQI to lipids *in vitro* as well as *in vivo* has received some attention with respect to hazard assessment of the use of acetaminophen.

The last potential causative event for damage, that is, the nuclear effects that are observed experimentally. Mostly, low but chronic levels of exposure are studied with

respect to potential nuclear effects of acetaminophen. However, DNA effects as a result of high hepatotoxic doses of acetaminophen, leading to apoptosis **[51].**

2.4.2. Lipid peroxidation:

Free radicals are reactive species frequently generated during metabolism of many compounds. In turn, reactive oxygen species, such as superoxide anion ($O_2^{\bullet}$), and hydrogen peroxide (H_2O_2), are produced by free radicals as well as during normal aerobic respiration. Oxidative stress occurs when there is an imbalance between the production and removal of reactive oxygen species, due to their overproduction or a decrease in antioxidant defenses, and the cell defense systems are overwhelmed **[52].**

Several experimental models confirmed increased polyunsaturated fatty acids (PUFA) oxidation of hepatic mitochondria lipid peroxidation occurred **[53].**

Acetaminophen produces oxidative stress in two ways. The indirect mechanism involves the formation of the intermediate N-acetyl-p-benzosemiquinone imine (NAPSQI) radical during redox cycling of acetaminophen which in turn transfers an electron to molecular oxygen to form NAPQI and $O_2^{\bullet}$ which can then cause lipid peroxidation **[54].**

As mentioned above, one of the phenomena often observed in combination with oxidative stress is lipid peroxidation. Reactive oxygen species (hydrogen peroxide, superoxide anions, and hydroxyl radicals) are required for its initiation. However, reduction of NAPQI, which could occur in the presence of flavoproteins, followed by reoxidation by oxygen could give rise to superoxide anions with a consequent formation of reactive reduced oxygen species.

Even protein bound NAPQI was suggested to be liable to one electron reduction. Lipid peroxidation has been regarded to be an important initiation event in the toxicity mechanism of acetaminophen **[55].**

GSSG formed by the oxidation of GSH by GSH peroxidase during the interaction with reactive oxygen species, is used as a measure of lipid peroxidation or

oxidative stress in *vivo* **[56].** In support of LPO as an important mechanism of hepatotoxicity, treated isolated mouse and rat hepatocytes with acetaminophen and detected an increase in cell damage and malonyl dialdehyde (MDA) formation, a product of lipid peroxidation **[27].**

2.4.3. Covalent Binding to Proteins:

2.4.3.1. Acetaminophen binding proteins:

Events that produce hepatocellular death following the formation of acetaminophen protein adducts are poorly understood. One possible mechanism of cell death is that covalent binding to critical cellular proteins results in subsequent loss of activity or function and eventual cell death and lysis. Primary cellular targets have been postulated to be mitochondrial proteins, with resulting loss of energy production, as well as proteins involved in cellular ion control and alterations of plasma membrane ATPase activity following toxic doses of acetaminophen **[57].**

The relationship between covalent binding of acetaminophen to hepatic proteins and the development of hepatotoxicity treated with a toxic dose of acetaminophen. Adducts of acetaminophen were observed immunohistochemically in the innermost layers of cells surrounding the central hepatic vein as early as 15 min. following a hepatotoxic dose of acetaminophen, by 30 min. there was a 90% depletion of hepatic glutathione and acetaminophen protein adducts were evident in the centrilobular area, by 1 h the protein adducts reached their maximum extent and were found exclusively in the centrilobular region of the liver by 2 h vacuolization and shrinking of hepatocytes were prominent**[58].**

2.4.3.2. Cytosolic binding proteins:

The first reported specific hepatic proteins in cytosol becoming arylated on *in vivo* exposure of mice to hepatotoxic doses of acetaminophen were a 52-kDa protein, a 54- kDa protein and a 100-kDa protein **[59].** These "acetaminophen binding proteins"

appeared to be native in many tissues, although adduct formation was only found in tissues that were prone to acetaminophen -based damage **[60].**

Selenium binding protein was identified as 52-kDa protein the function of selenium binding proteins is not known, but they were thought to be involved in a defense mechanism against arylating agents, because selenium binding proteins are common targets for electrophilic metabolites. Pretreating mice with selenium prior to acetaminophen administration decreased the total covalent binding in the liver, as well as arylation of the acetaminophen binding protein. The 54-kDa protein has been identified as cytosolic aldehyde dehydrogenase **[58,59].** This enzyme serves to reduce NAD to NADH so inhibition of this protein may have a detrimental effect on the redox status of the cell **[61].**

One group of researchers recently identified a 100- kDa cytosolic protein as a major target of acetaminophen and subsequently identified it as N-10-formyltetrahydrofolate dehydrogenase. A toxic dose of 400 mg/kg to mice resulted in a 25% decrease in cytosolic N-10-formyltetrahydrofolate dehydrogenase activity at 2 h already **[62].**

2.4.3.3. Mitochondrial binding proteins:

Besides cytosolic proteins, mitochondrial proteins also have been reported recently to become arylated after a hepatotoxic dose of acetaminophen.

Glutamate dehydrogenase (50-kDa), aldehyde dehydrogenase (56-kDa) were identified as being adducted after administration of hepatotoxic doses of acetaminophen **[42].** And ATP synthetase α-subunit (59-kDa) **[59].** Covalent modification of these mitochondrial proteins may result in impaired functioning of these proteins, which could give rise to impaired detoxification (oxidation) of aldehydes to acids, leading to lipid peroxidation. This could ultimately lead to mitochondrial damage as observed in hepatocytes as well as *in vivo* in mice as early as 1 h following acetaminophen administration **[63].**

The 50-kDa mitochondrial protein adducts as glutamate dehydrogenase **[64].** This enzyme reversibly metabolizes glutamate to α-ketoglutarate and ammonia. The activity of this enzyme was also decreased approximately 25% at 2 h. Covalent binding with acetaminophen resulted in partial inhibition of enzyme activity under toxic conditions in mice. Although it is plausible that partial inhibition of a large number of enzymes may contribute to cell death, the data generated questions relative to the validity of the hypothesis that covalent binding to critical proteins is the only mechanism of acetaminophen toxicity **[57].**

The second protein has been identified is 56-kDa protein which known as mitochondrial aldehyde dehydrogenase **[61].** Many of mitochondrial proteins are modified is consistent with the occurrence of mitochondrial dysfunction reported during the early stage of acetaminophen toxicity.

ATP synthetase α-subunit was identified as a (59-kDa), which consider as an essential subunit of the F_1 unit of ATP synthase. F_1 contains the catalytic site for ATP synthesis and modification of ATP synthetase α-subunit may have abolished the function of ATP synthase and subsequently resulted in ATP depletion [59]. The activity of ATPase decrease until to 35% after acetaminophen treatment (650 mg/kg) in rats and suggested this was due to covalent modification on ATPase by NAPQI [65].

2.3.3.4- Cytosolic and mitochondrial binding proteins:

Two different proteins were identified in the 22-kDa and 23-kDa regions. The first protein is Glutathione peroxidase (22-kDa) (GPx) is an enzyme that catalyzes the reduction of hydroperoxide into O_2 and water, together with the oxidation of GSH to GSSG. This reaction is a part of the mechanism that protects cells from oxidative damage induced by endogenous reactive oxygen species derived from superoxide anion, which may be released from the mitochondrial respiration chain. Earlier studies indicating that inhibition of glutathione peroxidase increased the susceptibility of hepatocytes to acetaminophen toxicity [66].

The second one is Glutathione *S*-transferase Pi (23-kDa) GST Pi. GSTs are a family of detoxification enzymes that catalyze the nucleophilic attack of the sulfur atom of glutathione over a wide range of electrophilic compounds such as NAPQI. The GST Pi class is expressed at high levels in tumors, and it has become clear that over expression of GST Pi plays a role in acquired resistance to chemotherapy. Covalent modification and inhibition of GSTs by various agents, a cytotoxic lipid peroxidation product, have been reported. Therefore, in retrospect, it is not surprising that GST is a target of NAPQI after GSH depletion **[59].** Elevated GST Pi has been associated with protection of liver cells from the cytotoxicity produced by acetaminophen **[67].**

2.4.4. Mitochondrial dysfunction and superoxide formation:

Several xenobiotics interact with the mitochondrial electron transport chain, increasing the rate of $O_2^{-\cdot}$ production through two different mechanisms. Some of these compounds stimulate oxidative stress because they block electron transport, increasing the reduction level of carriers located upstream of the inhibition site. Other xenobiotics may accept an electron from a respiratory carrier and transfer it to molecular oxygen (redox cycling), stimulating $O_2^{-\cdot}$ formation without inhibiting the respiratory chain **[49].**

Superoxide may be formed via a number of mechanisms including formation from cytochrome P_{450} in acetaminophen toxicity. This sudden excess utilization of oxygen by activated phagocytes is a result of increased activity of the enzyme, NADPH oxidase. The result is release of superoxide anion at the outer surface of the plasma membrane **[53].** Addition of NAPQI to isolated rat liver mitochondria caused a decrease in synthesis of ATP and this is consistent with the hypothesis that NAPQI causes MPT, as has been reported about other quinines. This is presumably a result of NAPQI mediated oxidation of the vicinal thiols at the MPT pore. NAPQI is known to be both an oxidizing agent and an arylation agent, the acetaminophen toxicity leads to oxidation of protein thiols **[68].** According to the toxicity of acetaminophen which occur by the oxidation of thiol groups in mitochondrial by the action of NAPQI leading to MPT the toxicity is mediated by mitochondrial dysfunction resulting in increase of reactive oxygen / nitrogen species where known as free radicals**[69].**

3. Free radicals and reactive species:

3.1. General:

Free radicals: are atoms or molecules that contain one or more unpaired electrons. Many radicals are highly reactive and can function as reducing or oxidizing agents by donating electrons to or removing electrons from other molecules. Small amounts of free radicals are constantly being generated in all living organisms. Although free radicals are potentially harmful to cellular components, a substantial body of evidence supports a role for these highly reactive chemical molecules in fundamental cellular reactions and cell-cycle regulation **[69].**

These free radicals are produced continuously by cells as part of normal cellular functions. About 95% of oxygen consumed by tissues is utilized in metabolic processes, but approximately 5% of the oxygen consumed is transformed into reactive species **[70].**

Our bodies are equipped with efficient enzymatic/non enzymatic antioxidant molecules that effectively detoxify the free radicals generated during various metabolism processes. However, under pathological conditions, an imbalance between production and detoxification of free radicals occur either due to increased production of reactive species their inefficient removal or both resulting in development of "oxidative stress" **[71].**

Accordingly, oxidants are being classified based on their chemical nature (radical or non radical), production site and reactivity towards biological targets. The radical group includes oxygen/nitrogen derived free radicals (ROS/RNS) such as superoxide, hydroxyl and nitric oxide, while the non-radical category includes metabolites of oxygen free radicals such as hydrogen peroxide, hypochlorous acid and, these free radicals being very reactive can potentially damage the biological architecture either alone or as a source for the generation of more reactive damaging species **[72].**

ROS/RNS are known to play a dual role in biological systems, since they can be either harmful or beneficial to living systems. Beneficial effects of ROS involve

physiological roles in cellular responses for example in defense against infectious agents and in the function of a number of cellular signaling systems **[73].**

3.2. Chemistry and biochemistry of ROS:

Reactive oxygen species (ROS) are small, highly reactive, oxygen-containing molecules that are naturally generated in small amounts during the body's metabolic reactions and can react with and damage complex cellular molecules such as fats, proteins, or DNA **[74].** The cellular toxicity of oxygen has been attributed to its affinity in accepting electrons from other molecules. Reactive oxygen species category includes both oxygen radicals/oxygen derived non radicals that possess high energy oxidizing potential **[72].**

3.2.1. Superoxide anion ($O_2^{-\cdot}$): Is a negatively charged free radical produced by a one electron reduction of molecular oxygen either by autooxidation or by the action of various oxidases. The most important reaction of superoxide radical is dismutation, where two superoxide molecules undergoes redox reaction, where one gets oxidized to oxygen and the other being reduced to hydrogen peroxide **[75].**

$$O_2 + e^- \xrightarrow{\text{oxidase}} O_2^{-\cdot}$$

3.2.2. Hydrogen peroxide (H_2O_2): Is a non-radical form of ROS formed as a result of dismutation of superoxide radicals. H_2O_2 is also synthesized in the reactions catalyzed by various oxidases. Although H_2O_2 is not a radical by definition, it also serves as a source for the generation of $^{\cdot}OH$ and HClO either by reacting with superoxide anion (Haber-Weiss reaction) or with free iron (Fenton reaction) **[76].**

3.2.3. Hydroxyl radical ($^{\cdot}OH$): The hydroxyl radical is highly reactive with a half-life in aqueous solution of less than 1 ns. Thus when produced *in vivo* it reacts close to its site of formation. It can be generated through a variety of mechanisms. Ionizing radiation causes decomposition of H_2O, resulting in the formation of $^{\cdot}OH$ and hydrogen atom. $^{\cdot}OH$ is also generated by photolytic decomposition of alkyl hydro peroxides. Production of $^{\cdot}OH$ close to DNA could lead to this radical reacting with DNA bases or

the deoxyribosyl backbone of DNA to produce damaged bases or strand breaks. Hydroxyl radical is formed *in vivo* when metal ions such as iron, copper, chromium and cobalt react with hydrogen peroxide "Fenton reaction" and also by "Haber-Weiss reaction".

$$O_2^{\bullet-} + H_2O_2 \longrightarrow O_2 + {}^{\bullet}OH + OH^- \quad \textit{"Haber-Weiss reaction"}$$

$$Fe\ (II) + H_2O_2 \longrightarrow Fe\ (III) + {}^{\bullet}OH + OH^- \ \textit{"Fenton reaction"}$$

3.2.4. Peroxyl radical ($ROO^{\bullet}$): The simplest peroxyl radical is the dioxyl (hydroperoxyl) radical $HOO^{\bullet}$. The chemistry of this type of molecule varies according to the nature of the R group **[73].** Perhaps the most interesting feature of peroxyl radicals is the diversity of those biological reactions in which they participate. The detection and measurement of lipid peroxidation is most frequently cited as evidence to support the involvement of peroxyl radical reactions in human disease and toxicology **[77, 78].**

3.3. Chemistry and biochemistry of RNS:

Various biochemical pathways may cause the formation of $NO_2^{\bullet}$ *in vivo*, including autoxidation of $NO^{\bullet}$ by reaction with O_2 (although this is presumed to be relatively insignificant at physiologic levels of $NO^{\bullet}$). Furthermore, NO_2^- can be protonated under acidic conditions to nitrous acid (HNO_2), and thereby induce both nitrosation and nitration of endogenous proteins **[79].**

3.3.1. Nitric oxide NO : Is produced by the oxidation of one of the terminal guanido nitrogen atoms of L-arginine by Nitric Oxide Synthase (NOS) that requires NADPH flavin nucleotides (FMN/FAD) tetrahydrobiopterin, calmodulin, and calcium as cofactors for its activity **[80].**

$$\textbf{L-Arginine + NADPH} \longrightarrow \textbf{L-citrulline + NO}$$

An abundant reactive radical that acts as an important oxidative biological signaling molecule in a large variety of diverse physiological processes, including defense mechanisms, smooth muscle relaxation, and immune regulation **[81,82].**

3.3.2. Peroxynitrite anion $ONOO^-$: Is produced via the diffusion-limited reaction of NO with the superoxide radical Peroxynitrite is readily protonated under physiological conditions. The protonation of peroxynitrite generates peroxynitrous acid (ONOOH). Peroxynitrite can also react with CO_2 to form a reactive CO_2 adduct of peroxynitrite, nitrosoperoxycarbonate, $ONOOCO_2^-$ which can react with tyrosine to give nitro-tyrosine and bicarbonate or decompose into nitrogen dioxide and carbonate radicals. The reaction of tyrosine with the nitrogen dioxide and carbonate radicals can also give the nitrotyrosine **[82].**

$$NO^{\bullet} + O_2^{-\cdot} \longrightarrow ONOO^-$$

Generation of oxidative compounds is physiologically relevant as an important step in inflammation and, therefore, it represents part of defense mechanisms against invading microorganisms. The damage of the tissues that occurs through a number of biochemical mechanisms, all of which have in common the formation of highly reactive compounds "free radical" that can oxidize protein, lipid, and nucleic acids.

According to the imbalance between these reactive molecule "reactive species" and defense system of the body i.e. "Antioxidant" the oxidative stress occurs **[83].**

4. Oxidative stress:

Oxidative stress is defined as the tissue damage resulting from an imbalance between an excessive generation of oxidant compounds free radical (ROS/RNS) and insufficient antioxidant defense mechanisms **[84].** Generation of oxidative compounds is physiologically relevant as an important step in inflammation process. It represents part of the defense mechanisms against invading microorganisms and malignant cells and each cell in the human body maintains a condition of homeostasis between the oxidant and antioxidant species **[85].**

On the other hand, an improper activation of oxidative processes may be chronically present in pathological situations, contributing to cell and tissue injury **[86].**

Oxidative stress initiated by free radicals, play a vital role in damaging various

cellular macromolecules. These include DNA molecules, proteins and lipids. This damage may result in neurodegenerative diseases and carcinogenesis **[87].**

4.1. Causes of oxidative stress:

Oxidative stress results from an imbalance between antioxidant defense mechanisms and excessive generation of oxidants, leading to cell and tissue injury, there is a deficiency in antioxidant systems (vitamin C and selenium deficiency, reduced intracellular vitamin E and activity of GSH system). At the same time, prooxidant activity is increased due to advanced age, diabetes, chronic inflammation. Tissue damage occurs through a number of biochemical mechanisms, all of which have in common the formation of highly reactive intermediate compounds (free radicals) that can oxidize proteins, lipids and nucleic acids **[83].**

4.2. Sources of oxidative stress:

The mitochondrial respiratory chain represents the most powerful cellular source of oxidants in the body. Mitochondrial oxidants may exert deleterious effects and are thought to contribute to cellular senescence, as well as neurodegenerative diseases. However, to date, there is no method available to determine their potential contribution to cellular pathology. The phagocyte oxidant generation system is based on the inducible production of reactive oxygen species (ROS) via univalent reduction of molecular oxygen (O_2): following exposure to appropriate stimuli, both polymorphonuclear neutrophils (PMNs) and monocyte macrophages activate and increase their O_2 consumption. The NADPH-oxidase enzyme system, which is bound to cellular membranes, reduces O_2 to superoxide anion (O^{-}_2), which is highly unstable and, as soon as it is formed, is converted into hydrogen peroxide (H_2O_2) **[83].**

Both O^{-}_2 and H_2O_2 are precursors for the production of more powerful oxidants. O^{-}_2 interacts with nitric oxide (NO) to form highly reactive nitrogen species (nitrosative stress), while H_2O_2 reacts with intracellular iron to form hydroxyl radicals (^{-}OH), that are heavily implicated in cell membrane lipid degradation, protein aggregation and DNA damage. H_2O_2 is the substrate for myeloperoxidase (MPO) to produce the chlorinated oxidants. In the presence of Cl^{-}, MPO converts H_2O_2 into hypochlorous acid (HOCl), a powerful compound capable of oxidizing a number of molecules, such as

lipids, proteoglycans and other membranous or intracellular constituents, particularly the thiol groups of membrane proteins (chlorinative stress). In addition, it may react with endogenous amines (R-NH2) to produce chloramines (RNH-Cl).

The ROS are released together with pro inflammatory cytokines, which in turn amplify oxidant generation **[88].**

4.3. Markers of oxidative stress:

Oxidative stress can result in injury to all the important cellular components like proteins, DNA and cell membrane lipids, which can cause cell death. Moreover, there are several experiments and clinical data providing compelling evidence for the involvement of oxidative stress in large number of pathological states including carcinogenesis **[89].** Oxidants are highly reactive compounds with a half life of only seconds. Therefore, their in vivo determination is generally not feasible. In contrast lipids, proteins, carbohydrates and nucleic acids, after being modified by oxy radicals, have lifetimes ranging from hours to weeks, which make them ideal markers of oxidant stress **[90].**

The degree of haemolysis can indirectly reflect oxidative stress, since oxidation of plasma membrane proteins can reduce membrane elasticity and erythrocyte survival **[83].**

During lipid peroxidation, unstable hydroperoxides, resulting from peroxyl radical-dependent chain reactions among unsaturated fatty acyl moieties, break down to smaller and more stable products, e.g. aldehydes, such as acrolein, malonyldialdehyde (MDA) **[86].**

Determination of the 8-hydroxy-2□- deoxyguanosine (8-OHdG) content by high performance liquid chromatography (HPLC) was used to evaluate DNA damage. Oxidative stress has been demonstrated to be involved in various physiological and pathological processes, including DNA damage. Oxidative compounds may interact with nucleic acids and contribute to mutagenesis and oncogenesis **[91].**

Aerobic organisms have adapted for existence under continuous exposure to ROS. Among the various adaptive mechanisms, the cellular antioxidant defense system plays a major role in removal of prooxidants **[92].**

The antioxidants are of great interest as possible protective agents to help the human body reduces oxidative damage without any interference **[93].**

5. ANTIOXIDANTS :

5.1. General:

Antioxidants: can be defined as substances whose presences in relatively low concentrations significantly inhibit the role of oxidation of the target cells. Due to continuous generation of partially reduced forms of oxygen by constitutive metabolic pathways, a number of protective antioxidant enzyme, such as SOD, CAT, GPx and non- enzymatic antioxidants such as vitamin C, vitamin E have involved to deal with toxic species **[94].** Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical-induced oxidative stress. A variety of free radical scavenging antioxidants is found in a number of dietary sources **[95].**

The health-promoting properties of fruits and vegetables are due to the presence of some vitamins (A, C, E) and phytochemicals in these food products. Among phytochemicals, polyphenols deserve a special mention due to their free radical scavenging activities and *in vivo* biological activities that are being investigated by many researchers. In the past few years there has been a renewed interest in studying and quantifying the phenolic metabolites of fruits and vegetables due to their health-promoting properties. Fruit polyphenols include a wide range of compounds with antioxidant activity **[96].**

Antioxidants refer to compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. They act in one or more of the following ways as reducing agents, by free radical scavenging, and as quenchers of singlet oxygen **[97].** Some antioxidants act in a hydrophilic environment, others in a hydrophobic environment, also there is type of antioxidants act in both environments of the cell. For example, vitamin C reacts with superoxide in the aqueous phase while vitamin E does so in the lipophilic phase. In

contrast, lipoic acid is both water and fat soluble and therefore can operate both in cellular membranes and in cytosol **[98].**

Antioxidant molecules can be divided into different categories based on their functions (enzymatic or nonenzymatic), their physical properties (water-soluble or lipid-soluble) and their sources (endogenous or exogenous).

5.2. Enzymatic antioxidants:

5.2.1. Superoxide dismutase SOD:

Catalyzes the spontaneous dismutation of superoxide into hydrogen peroxide. The enzyme exists in two forms, a manganese containing SOD (Mn-SOD, mitochondria) and a copper-zinc dependant (Cu-Zn SOD, cytoplasm). These enzymes are the first line of defense against oxidative stress **[99].**

$$\text{Enzyme} - Cu^{2+} + O_2^{\bullet -} \xrightarrow{\text{SOD}} \text{Enzyme} - Cu^{1+} + O_2$$

$$\text{Enzyme} - Cu^{1+} + O_2^{\bullet -} + 2H^+ \xrightarrow{\text{SOD}} \text{Enzyme} - Cu^{2+} + H_2O_2$$

5.2.2. Catalase (CAT):

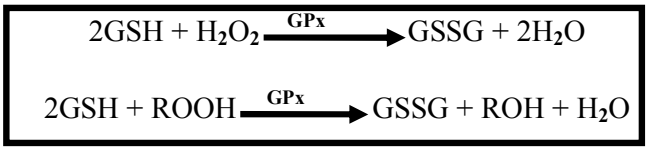
Is an enzyme presents in the cells of plants, animals and aerobic (oxygen requiring) bacteria. Catalase is located in a cell organelle called the peroxisome. The enzyme very efficiently promotes the conversion of hydrogen peroxide to water and molecular oxygen. One molecule of catalase can convert ~6 million molecules of hydrogen peroxide to water and oxygen each minute **[100].**

$$2H_2O_2 \xrightarrow{\text{CAT}} 2H_2O + O_2$$

5.2.3. Glutathione peroxidase (GPx):

Glutathione is one of the most essential of antioxidative defense mechanisms. Humans have four different Se-dependent glutathione peroxidases **[101].** All GPx enzymes are known to add two electrons to reduce peroxides by forming selenoles (Se-

OH). The antioxidant properties of these selenoenzymes allow them to eliminate peroxides as potential substrates for the Fenton reaction. The substrate for the catalytic reaction of GPx is H_2O_2, or organic peroxide ROOH. GPx decomposes peroxides to water (or alcohol) while simultaneously oxidizing GSH **[100].**



5.2.4. Glutathione reductase (GR):

An enzyme belonging to the flavoprotein disulfide oxidoreductase family. It reduces GSSG to GSH at the expense of NADPH, which is provided via the glucose monophosphate shunt. The reaction of converting GSSG to GSH is fast and irreversible, thus the enzyme can provide cells with a high intracellular GSH/GSSG ratio **[102].**

5.3. Non-enzymatic antioxidants:

Nonenzymatic antioxidant which divided into lipophilic and hydrophilic antioxidants and also include low molecular mass agents synthesized *in vivo* also which derived from the diet such as vitamins.

5.3.1. Lipid-soluble antioxidants:

5.3.1.1. Vitamin E:

A fat soluble vitamin, also known as the α -tocopherol is the most active form of vitamin E in humans and is a powerful biological antioxidant which is considered to be the major membrane bound antioxidant employed by the cell **[103].** Its antioxidant action depends on the breaking of the propagation of free radical chains **[104].**

α-tocopherol is a free radical chain breaking antioxidant that acts by neutralizing a free radical gets converted to α-tocopheroxyl radical. It can be reduced back to α-tocopherol by ascorbic acid. Dehydroascorbic acid formed in this reaction can be reconverted back to ascorbic acid by reduced glutathione. It is important that sufficient amounts of α-tocopherol, reduced ascorbic acid and reduced glutathione be present within the cell so as to provide protection against oxidative injury **[105].**

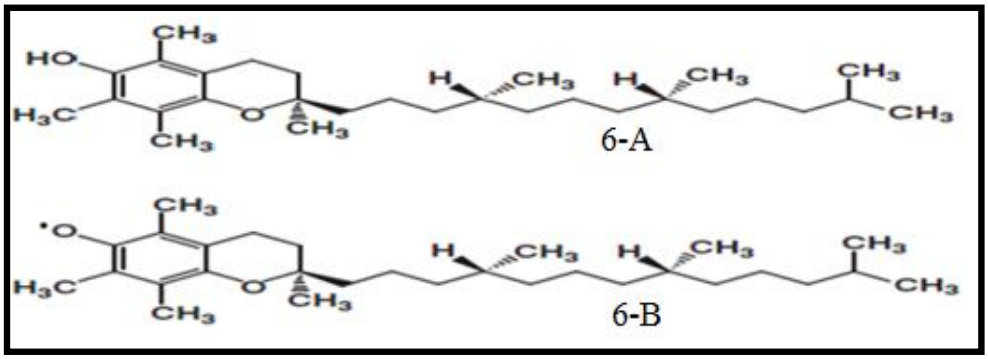


Fig. (6): **A**: α-Tocopherol, **B**: α- Tocopherol radical **"**Tocopheroxyl radical**"**

5.3.1.2. Ubiquinone:

It is a fat soluble quinone, also known as CoQ-10 (2,3-dimethoxy-5-methylbenzoquinone) fig. (7) is a naturally occurring quinone that is found in most aerobic organisms from bacteria to mammals **[106].** Physiologically, CoQ10 plays four major roles. It has an essential role in mitochondrial energy (ATP) production through redox activity in the respiratory chain, transporting electrons between enzymes. The another role in extramitochondrial redox activity in the cell membrane and endomembranes. CoQ10 also functions as an antioxidant, inhibiting lipid peroxidation and scavenging free radicals. Finally, it plays an important role in membrane stabilization and fluidity. It prevents free radical oxidation of low density lipoprotein (LDL) and very low density lipoprotein (VLDL). Pretreatment with CoQ10 provided significant protection against oxidant induced cell injury **[106].**

Ubiquinol contains a redox active group benzoquinol attached to a hydrophobic side chain. This hydrocarbon side chain is composed of multiple five-carbon isoprene

units, and the most common form of ubiquinol in humans contains a side chain with ten isoprene units **[102].**

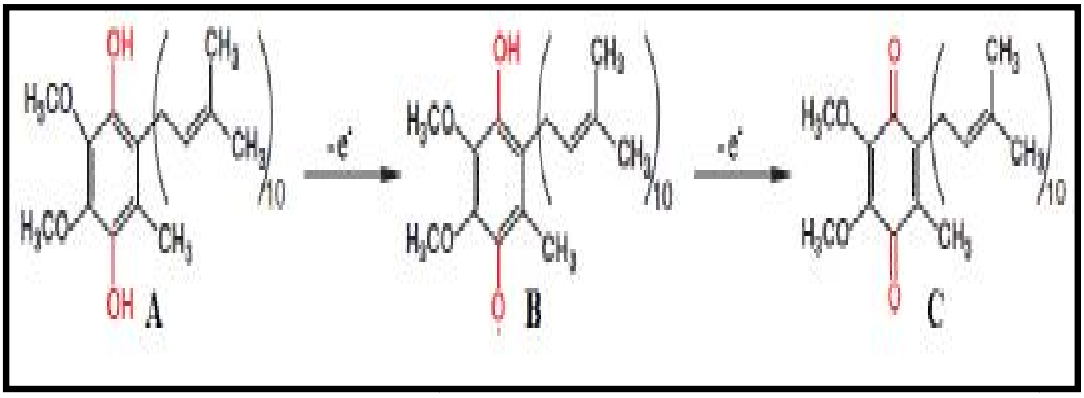


Fig. (7) : **A**: Ubiquinol, **B**: Semiquinol, **C**: Ubiquinone

5.3.2. Water-Soluble Antioxidants:

5.3.2.1. Vitamin C:

Ascorbic acid is a very important and powerful antioxidant that works in aqueous environments of the body. Its primary antioxidant partners are vitamin E and the carotenoids, as well as working along with the antioxidant enzymes. Vitamin C cooperates with vitamin E to regenerate α-tocopherol from α-tocopheroxyl radicals in cell membranes and lipoproteins **[107,108].**

Ascorbic acid has two ionisable hydroxyl groups and therefore is a di-acid ($AscH_2$). At physiological pH, 99.9% of vitamin C is present as $AscH^-$, and only very small proportions as $AscH_2$ (0.05%) and Asc^{2-} (0.05%). The antioxidant chemistry of vitamin C is thus the chemistry of $AscH^-$. $AscH^-$ is a donor antioxidant and reacts with radicals to produce the resonance stabilized tricarbonyl ascorbate free radical ($Asc^{\bullet-}$). It is not protonated but is present in the form of $Asc^{\bullet-}$. Thus the product of ascorbate oxidation by many ROS is the semidehydroascorbate radical ($Asc^{\bullet-}$) a poorly reactive radical that is considered to be a terminal **[109,110].**

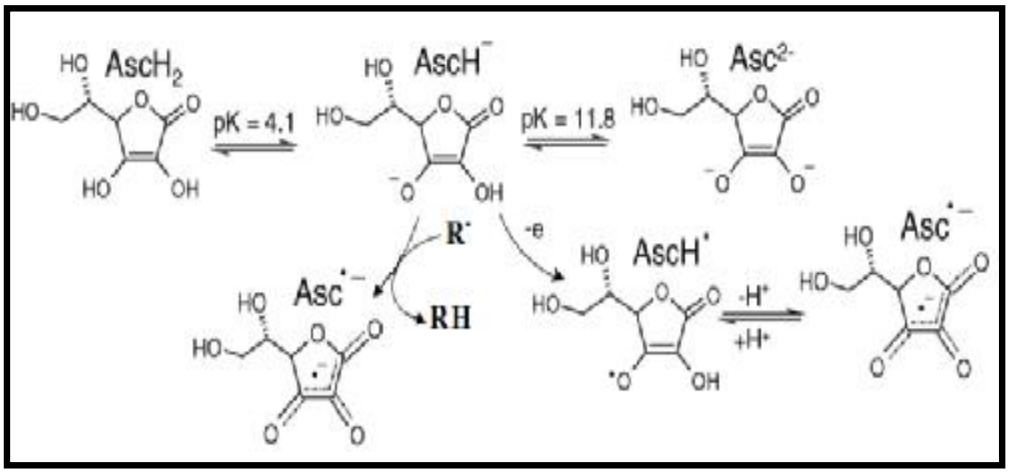


Fig. (8): Various forms of ascorbic acid (vitamin C) and its reaction with radicals (R•)

Its antioxidant capacities have raised a great deal of interest in the role of ascorbate in human health and disease. For example, oxidative damage to DNA is believed to be an important cause underlying cancers, and increased levels of ascorbate in cell cultures have proved effective in reducing free radical induced mutation rates. However, a large number of the physiological effects of ascorbate are actually mediated by its role as a cofactor for a variety of redox enzymes.

This illustrates that the role of ascorbate in preventing disease is clearly attributed to its function as a free radical scavenger **[102].** In the cytosol, ascorbate acts as a primary antioxidant to scavenge free radical species that are generated by products of cellular metabolism. At cellular membrane levels, it may play an indirect antioxidant role to reduce the α-tocopheroxyl radical to α-tocopherol. Recycling of α-tocopherol by ascorbate has been demonstrated in liposomes and cellular organelles **[111].**

5.3.2.1. Uric acid:

It is a modestly water soluble antioxidant with the ability to neutralize a broad spectrum of ROS, particularly singlet oxygen and free radicals. Uric acid is an end product of purine catabolism and is one of the most abundant circulating antioxidants **[102].**

Uric acid is produced from hypoxanthine and xanthine by xanthine oxidase and xanthine reductase enzymes. In most species, the H_2O_2 producing peroxisomal enzyme urate oxidase converts it to allantoin, which is further convert to allantoate and then gloxylate plus urea, all products much more soluble in water than is urate, when the activity of the urate oxidase is absent and urate accumulates in blood plasma to high concentrations**[112].**

The urate is a powerful scavenger of ROS in vitro, it can function as biological antioxidant and further suggested that loss of urate oxidase was advantageous to primates since it simultaneously removed a source of H_2O_2 and allowed a powerful antioxidant to accumulate Where the urate acts as powerful scavenger for $NO_2^{\bullet}$ and may help to protect biomolecules against these oxidizing air pollutants and also protects proteins against nitration **[113].**

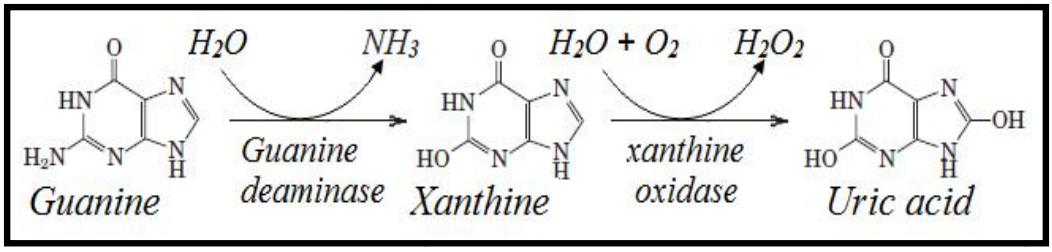


Fig. (9): Pathway of uric acid formation

5.4. Phenolic antioxidants:

Recent interest in phenolic compounds in general and flavonoids in particular, has increased greatly owing to their antioxidant capacity and their possible beneficial implications in human health. Phenolic compounds acting as antioxidants may function as terminators of free radical chains and as chelators of redox-active metal ions that are capable of catalyzing lipid peroxidation **[114].**

Flavonoids are a large group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, roots, stems, flowers, and leaves of the majority of plant. These natural products were known for their beneficial effects on health before flavonoids were effectively isolated as pure compounds. Many of which are responsible for the attractive colors of flowers, fruit, and leaves **[115].**

Flavonoids are divided into many chemical classes such as flavones, flavanones, flavanols and flavonols fig. (10) and many others.

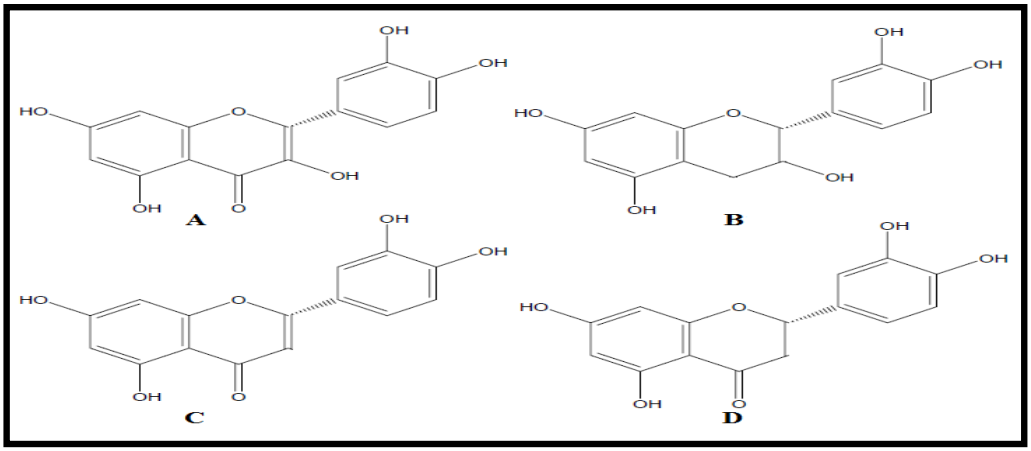


Fig. (10): A- Flavonols, B- Flavanols, C- Flavones, D- Flavanones.

These compounds have long been recognized to possess antihepatotoxic, anti-inflammatory, antiatherogenic and anticancer activities. Many of the pharmacological effects of flavonoids are related to their interaction with several enzymes and to their antioxidant activity, which can be due to their ability to scavenge free radicals, to their chelation of metal ions and to their synergistic effects with other antioxidants **[116].**

The major effects of flavonoids may be the result of radical scavenging. Another possible mechanism by which flavonoids act is through interaction with various enzyme systems. Furthermore, some effects may be a result of a combination of radical scavenging and an interaction with enzyme functions **[117].**

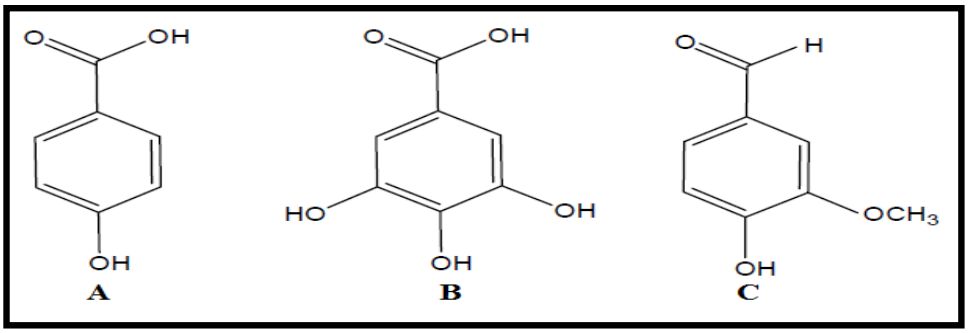


Fig. (11): **A**: p-hydroxybenzoic acid, **B**: gallic acid, **C**: vanillic acid

MATERIALS AND METHODS

1. Materials:

1.1. *Ecballium elaterium* "fruit juice":

The ripe fruits of *Ecballium elaterium* were collected from Benghazi city "Shibna land" during late June 2012.

1.2. Chemicals:

1,1-Diphenylpicrylhydrazyl radical (DPPH˙), and silymarin, ascorbic acid, Folin-Ciocalteu reagent, ferric chloride, potassium ferricyanide, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, trichloro acetic acid ,sodium carbonate, quercetin and pyrogallol were purchased from Sigma company for Chemicals.

1.3. Experimental animals:

A total of 63 adult male albino rats weighting 90-120 g were used in this study. Rats were provided from the animal house in faculty of medicine university of Benghazi and the rats were acclimatized to laboratory condition for 10 days before commencement of experimental. Animals allowed free access of water and fed on a standard diet according to National Advisory Committee for Laboratory Animal Research **N.A.C.L.A.R**., 2004 **[118].**

2. Methods:

2.1. Preparation of *Ecballium elaterium* " fruit juice":

The fruits of *Ecballium elaterium* are well crushed. The obtained crude juice were refined by filtration using Whatman filter paper No.4 the refined crude juice is used in the estimation of antioxidant activity and to study its effect in treatment of hepatotoxicity.

The LD_{50} of fruit extract of *Ecballium elaterium* plant was nearly equal to 57.5 mg/kg. The maximal tolerated dose of fruit extract of *Ecballium elaterium* plant 20 mg/kg in mice **[119].**

2.2. Antioxidant activity and quantitative analysis assays.

2.2.1. Total phenolic content (TPC):

Total phenolic content was estimated using the colorimetric method based on Folin-Ciocalteu reagent **[120].** (100,200,300,400,500 µl) of *E. elaterium* "fruit juice" was diluted by 2ml of distilled water and mixed with "600 µl" of Folin-Ciocalteu reagent the mixture was allowed to stand for 5 min. and then 2 ml of 20% Na_2CO_3 was added and kept in boiling water bath for 1 minute, after cooling the blue colour was measured at 765 nm by UV-visible spectrophotometer. Quantification was done with respect to stander calibration curve of pyrogallol the results were expressed as pyrogallol "µg/ml".

2.2.2. Total flavonoid content (TFC):

Aluminum chloride colorimetric method was used for flavonoids determination **[121].** (100,200,300,400,500 µl) of *E. elaterium* "fruit juice" mixed with 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min., the absorbance of the reaction mixture was measured at 415 nm with a UV-visible spectrophotometer. The calibration curve was prepared by preparing quercetin solution in methanol at concentrations (100 to 500 µg/ml).

2.2.3. Reducing power assay (RPA):

Reducing power assay **[122].** was carried out by mixing (100,200,300,400,500 µl) of *E. elaterium* "fruit juice" with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide then mixture was incubated in water bath at 50 ^{0}C for 20 minutes and 2.5 ml of trichloroacetic acid was added to the mixture which was then centrifuged at 3000 rpm for 10 minutes. Finally 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 1 ml $Fecl_3$ and absorbance of prussian blue colour measured at 700 nm by UV-visible spectrophotometer. Quantification was done with

respect to stander calibration curve of ascorbic acid the results were expressed as ascorbic acid (µg/ml).

2.2.4. DPPH$^{\cdot}$ quenching activity:

Solution of DPPH$^{\cdot}$ (0.2 mM) in methanol was prepared by dissolving 0.008 mg of DPPH$^{\cdot}$ in methanol and volume was made up to 100 ml with methanol **[123].** The DPPH$^{\cdot}$ scavenging activity of the *E. elaterium* "fruit juice" was estimated by mixing (100, 200,300, 400, 500 µl) of *E. elaterium* "fruit juice" with 2 ml of 0.2 mM DPPH$^{\cdot}$ in methanol was kept in darkness at room temperature for 30 minutes the absorbance of the sample was measured at 517 nm by UV-visible spectrophotometer. Radical scavenging activity was expressed as percent of inhibition and was calculated using the following formula:-

%DPPH$^{\cdot}$ inhibition = [Abs. of Control - Abs. of Sample / Abs. of Control] x 100

2.3. Acetaminophen dose regimen:

Acetaminophen was purchased from Sigma Aldrich. The dose administered to the rat was set as 400 mg/kg. The powdered acetaminophen was dissolved in warm water and was administered according to the body weight of rat.

2.4. Induction of hepatotoxicity:

The toxic dose was chosen according to Roberts *et al.*, 1991 **[124].** Were recorded as 400 mg/ kg body weight of rats. The rats were acclimatized to laboratory condition for 10 days before commencement of experimental. Acetaminophen was orally administered to animals with dose 400 mg/kg at every 48 h for 22 days.

2.5. Experimental design:

2.5.1. The prophylactic effect of different treatments against hepatotoxicity:

To study the protective effect of *Ecballium elaterium* "fruit juice" against hepatotoxicity, a total of 28 rats were used and the experiment lasted for 3 weeks. Animals were divided randomly into four groups each group contain 7 rats as follows:

Group 1: Rats were fed on the standard diet and served as negative control (-ve) for 3 weeks.

Group 2: Rats were administered acetaminophen 400 mg/kg body weight orally every 48 h for 22 days; and served as positive control group (+ve).

Group 3: Rats were administered *Ecballium elaterium* "fruit juice" at a dose of 1 ml/kg orally before 1 hour of oral administration of acetaminophen.

Group 4: Rats were administered vitamin C at a dose of 300 mg/kg body weight orally before 1 hour of oral administration of acetaminophen.

2.5.2. The curative effect of different treatments on hepatotoxicity rats:

In this experiment, a total of 35 rats were used. 7 rats were fed on the standard diet and served as negative control (-ve) **group1**.

The other rats were subjected to the induction of experimental hepatotoxicity for 22 days where treated by acetaminophen at 400 mg/kg every 48 h and treated for 22 days.

The hepatotoxicity rats (28 rats) where divided randomly into equal 4 groups (each group contain 7 rats).

Group 2: Rats were served as hepatotoxicity animals (+ve).

Group 3: Rats were daily received *Ecballium elaterium* "fruit juice" at a dose of 1 ml/kg orally for 22 days.

Group 4: Rats were daily received vitamin C at a dose of 300 mg/kg body weight orally for 22 days.

Group 5: Rats were daily received silymarin at a dose of 50 mg/kg body weight orally for 22 days.

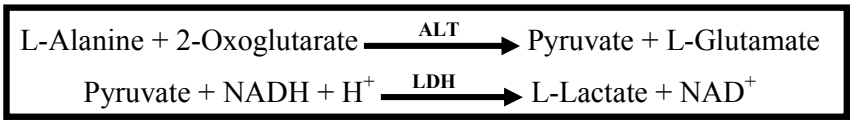
2.6. Blood sampling:

Blood samples were collected from rats before treatment and then after 3 weeks. Blood samples were obtained from the animal (in clean and dry centrifuge tubes) the tubes were centrifuged and clear serum and plasma was carefully withdrawn and kept in eppendrof tube for the determination the biochemical parameters.

2.7. Biochemical analysis:

2.7.1. Determination of alanine amino transferase (ALT):

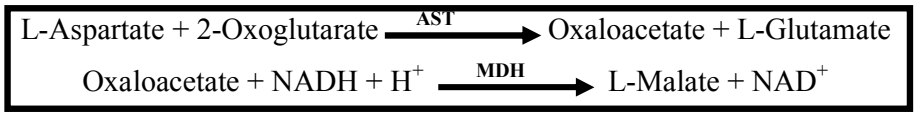
ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD^+. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.



The rate of NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance at 340 nm **[125].**

2.7.2. Determination of aspartate amino transferase AST:

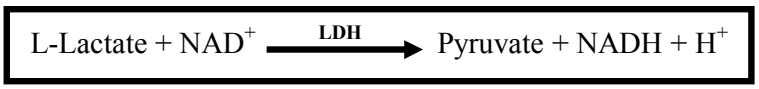
AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD^+. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction.



The rate of NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance at 340 nm **[126].**

2.7.3. Determination of serum lactate dehydrogenase (LDH):

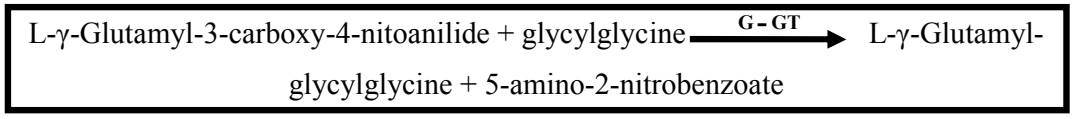
Lactate dehydrogenase catalyzes the conversion of L-lactate to pyruvate; NAD is reduced to NADH in the process.



The initial rate of the NADH formation is directly proportional to the catalytic LDH activity. It is determined by measuring the increase in absorbance at 340 nm **[127].**

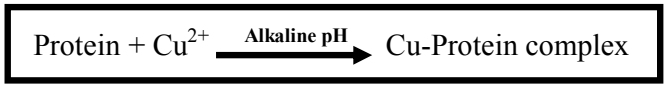
2.7.4. Determination of serum gama- glutamyl transferase (G –GT):

The amount of 5-amino-2-nitrobenzoate liberated is proportional to the G-GT activity in the sample. It is determined by measuring the increase in absorbance at 409 nm **[128].**



2.7.5. Determination of serum total protein:

Divalent copper reacts with the peptide bonds of proteins under alkaline conditions to form the characteristic pink to purple biuret complex. Sodium potassium tartrate prevents copper hydroxide precipitation and potassium iodide prevents the autoreduction of copper.



The color intensity is directly proportional to the protein concentration. It is determined by measuring the increase in absorbance at 552 nm **[129].**

2.7.6. Determination of serum albumin:

At pH 4.3, albumin is sufficiently cationic to bind the anionic dye bromcresol green (BCG) to form a blue-green colored complex.

$$\text{Albumin + BCG} \xrightarrow{\text{pH 4.3}} \text{albumin-BCG complex}$$

The intensity of the blue-green color is directly proportional to the concentration of albumin in the sample. It is determined by monitoring the increase in absorbance at 629 nm **[130].**

2.7.7. Determination of serum total bilirubin:

Since the absorbance maximum for azobilirubin is pH dependent, an oxalic acid/sulfanilic acid buffer system is used to maintain the pH of the reaction. The color intensity is proportional to the concentration of total bilirubin in the sample and is determined by monitoring the increase in absorbance at 552 nm **[131].**

$$\text{Sulfanilic acid + } NaNo_2 \xrightarrow{\text{HCl}} \text{diazotized sulfanilic acid}$$

$$\text{Bilirubin + diazotized} \xrightarrow{\text{pH 1.4}} \text{azobilirubin sulfanilic acid}$$

2.7.8. Determination of serum alkaline phosphatase (ALP):

In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol.

$$\text{AMP + 4-NPP} \xrightarrow{\text{ALP}} \text{4-nitrophenoxide + AMP-phosphate}$$

The p-ntirophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance at 409 nm **[132,133].**

2.7.9. Determination of serum creatinine:

In alkaline solution creatinine reacts with picrate to form a yellow-red adduct.

$$\text{Creatinine + picric acid} \xrightarrow{\text{Alkaline pH}} \text{yellow-red complex}$$

The rate of the dye formation is directly proportional to the creatinine concentration in the specimen. It is determined by measuring the increase in absorbance at 512 nm **[134,135].**

2.7.10. Determination of blood urea:

Urea is hydrolyzed by urease to form ammonium and carbonate. In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD.

$$\text{Urea} + 2\,H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-}$$

$$NH_4^+ + \text{2-oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 340 nm**[136].**

2.7.11. Determination of glutathione reductase (GR):

In method of enzymatic analysis glutathione reductase catalyses the reduction of glutathione (GSSG) in the presence of NADPH, which is oxidized to $NADP^+$. The decrease in absorbance at 340 nm is measured **[137].**

$$NADPH + H^+ + GSSG \xrightarrow{GR} NADP^+ + 2GSH$$

2.7.12. Determination of glutathione peroxidase (GPx):

Glutathione peroxidation (GPx) activity was determined by the assay is an indirect measure of the activity of GPx, oxidized glutathione (GSSG) produced upon reduction of organic peroxide by GPx, is recycled to its reduced state by the enzyme glutathione reductase (GR) **[138].**

$$\text{R-O-O-H} + 2\,GSH \xrightarrow{GPx} \text{R-O-H} + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + NADP^+$$

2.7.13. Determination of Catalase (CAT):

Catalase (CAT) activity was assayed colorimetrically. Catalase reacts with known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor **[139,140].**

2.7.14. Determination of superoxide dismutase (SOD):

Superoxide dismutase assay (SOD) activity was assayed according to the assay relies on the ability of enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye **[141].**

2.7.15. Determination of malondialdehyde (MDA):

According to Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95^0C for 30 min., to form thiobarbituric acid reactive product the absorbance of resultant pink product can be measured at 534nm **[142,143].**

2.8. Histopathological studies:

At the end of experiments, animals in all groups were scarified dislocation for histopathological studies, liver was removed and fixed in 10% neutral formalin. The slides were coded and were examined by a histopathologist in Annoon medical laboratory. After which photographs were taken.

2.9. Statistical analysis of the data:

The results obtained were statistically analyzed according to the methods described by the probability "P" was deduced from table of "t" test according to the degree of freedom. **[144].**

2.9.1. Arithmetic mean:

$(\bar{X}) = \sum \frac{(X)}{n}$ where; $(\bar{X})$ = Arithmetic mean; (X) = Sum of the individual values of the variant and n = Number of measurements.

2.9.2. Standard Deviation (S.D.):

S.D. = $\sqrt{\frac{\sum d^2}{n-1}}$ where

S.D. = standard deviation.

$\sum d^2$ = Sum of squared deviations of the individual values of the variant from the arithmetic mean ($\bar{X}$) of the series and n = Number of observations

2.9.3. Standard Error (S.E.):

S.E. = $\sqrt{\frac{\sum d^2}{n(n-1)}}$ where:

S.E. = standard error

$\sum d^2$ = Sum of squared deviations of the values of the variant from the arithmetic mean of series and n = Number of observation.

N.B. S.E. should not exceed 10% from the mean value and S.D. should not exceed 25% from the mean value.

"t" calculated = $\frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\sum d_1^2 + \sum d_2^2}{n_1 + n_2 - 2}\left[\frac{1}{n_1} - \frac{1}{n_2}\right]}}$ where

$\bar{X}_1 - \bar{X}_2$ = difference between the sample means.

$\sum d_1^2 + \sum d_2^2$ = stand for the sum of the squared deviations of the individual values of sample (1) and (2) respectively from their corresponding mean values.

n_1 and n_2 = number of observations in each of the two samples.

† Insignificant at P> 0.1; * Significant at P<0.05; ** Highly significant at P<0.01; *** Very highly significant at P<0.001.

RESULTS

1. Phytochemical screening of *Ecballium elaterium* "fruit juice"

Based on the absorbance values of the *Ecballium elaterium* "fruit juice" and compared with the standard solutions of pyrogallol, quercetin and vitamin C as described below.

Results obtained in table (1) and figs. (12, 13) referred to total phenolic content of *Ecballium elaterium* "fruit juice" where compared with pyrogallol as a standard phenolic compound.

Total flavonoids content also determined in fruit juice of *Ecballium elaterium* were compared with quercetin and as illustrated in table (2) and figs. (14, 15).

The reducing capacity of *Ecballium elaterium* "fruit juice" has been compared with the ascorbic acid according to the results that mentioned in table (3) and figs. (16, 17).

The results of the DPPH˙ radical scavenging activity of *Ecballium elaterium* "fruit juice" are shown in table (4) and fig. (18, 19).

These results are compared with the well known antioxidant ascorbic acid the percent of inhibition started from 37% at 100 µl to 89% at 500 µl and recorded from 44% at 100 µg/ml to 84% at 500 µg/ml in ascorbic acid.

Table (1): Total phenolic content (TPC) of *Ecballium elaterium* "fruit juice" compared to pyrogallol.

Volume of Ecballium elaterium "µl"	Mean ± Standard Deviation	Concentration of Pyrogallol "µg/ml"	Mean ± Standard Deviation
100	0.525 ± 0.0162	100	0.438 ±0.020
200	0.725 ± 0.020	200	0.725 ± 0.050
300	0.922 ± 0.022	300	1.070 ±0.087
400	1.154 ± 0.036	400	1.307 ±0.027
500	1.361 ± 0.037	500	1.564 ±0.075

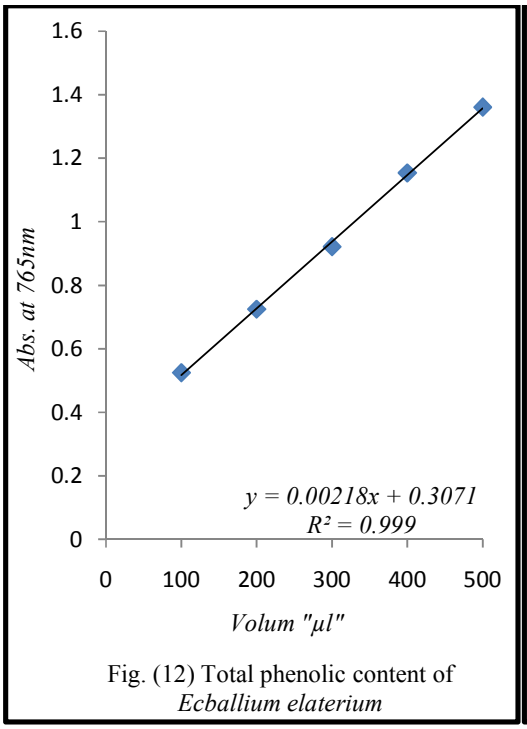


Fig. (12) Total phenolic content of *Ecballium elaterium*

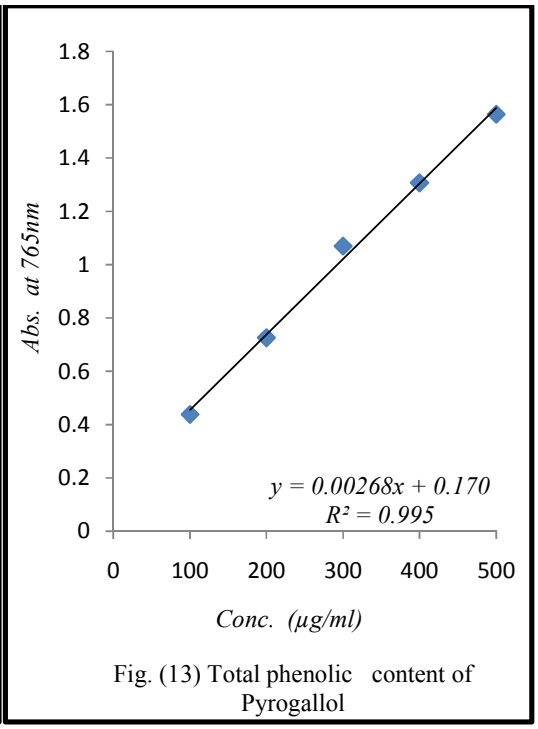


Fig. (13) Total phenolic content of Pyrogallol

Table (2): Total flavonoid content of *Ecballium elaterium* "fruit juice" compared to quercetin.

Volume of Ecballium elaterium "µl"	Mean ± Standard Deviation	Concentration of quercetin "µg/ml"	Mean ± Standard Deviation
100	0.0845 ± 0.055	100	0.307±0.025
200	0.135 ±0.045	200	0.587 ±0.075
300	0.191±0.075	300	0.974 ±0.074
400	0.223 ±0.071	400	1.203 ±0.056
500	0.266 ±0.085	500	1.511 ±0.026

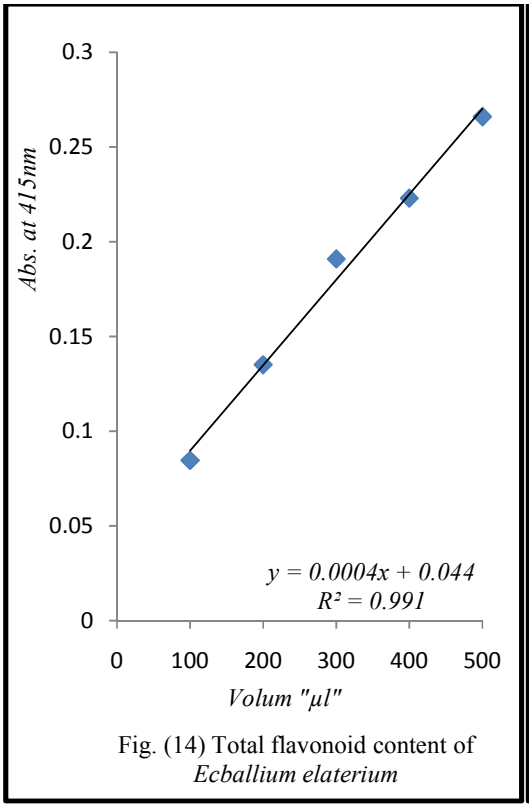


Fig. (14) Total flavonoid content of *Ecballium elaterium*

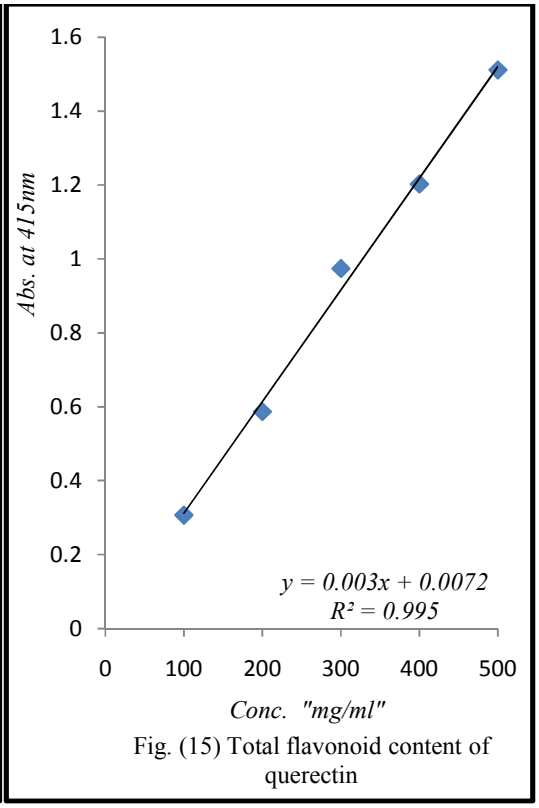


Fig. (15) Total flavonoid content of querectin

Table (3): Reducing power assay of *Ecballium elaterium* "fruit juice" compared to vitamin C.

Volume of Ecballium elaterium "µl"	Mean ± Standard Deviation	Concentration of vitamin C "µg/ml	Mean ± Standard Deviation
100	0.503 ±0.0448	100	0.201 ±0.0168
200	0.875±0.0965	200	0.495 ±0.0264
300	1.293 ±0.0471	300	0.697 ±0.0308
400	1.563 ±0.0266	400	0.992 ±0.0173
500	2.039 ±0.0401	500	1.201 ±0.0264

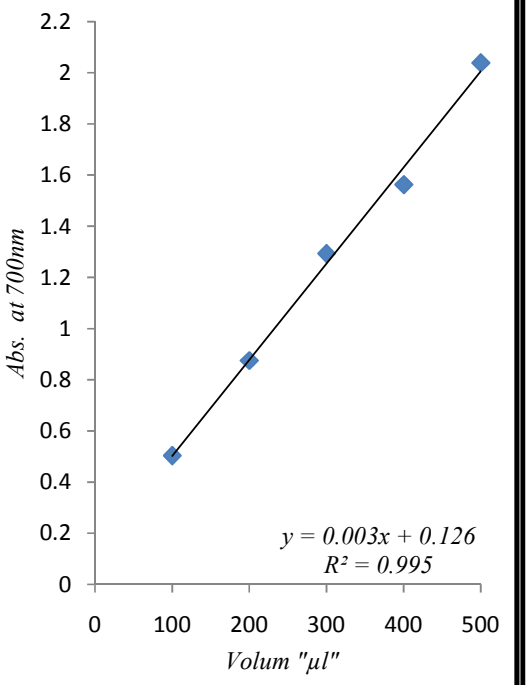


Fig. (16) Reducing power assay of *Ecballium elaterium*

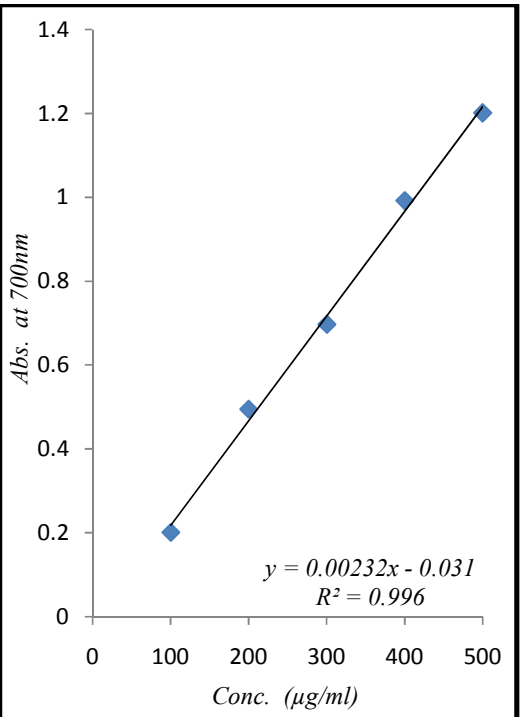


Fig. (17) Reducing power assay of vitamin C

Table (4): DPPH radical scavenging of *Ecballium elaterium* "fruit juice" compared to vitamin C.

Volume of Ecballium elaterium "µl"	Percent of inhibition %	Concentration of vitamin C "µg/ml	Percent of inhibition %
100	37%	100	44%
200	49%	200	54%
300	60%	300	67%
400	73%	400	76%
500	89%	500	84%

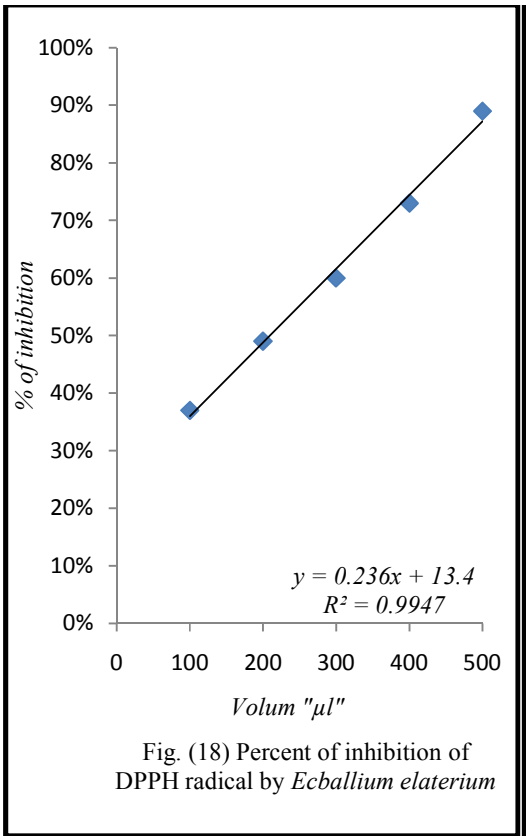


Fig. (18) Percent of inhibition of DPPH radical by *Ecballium elaterium*

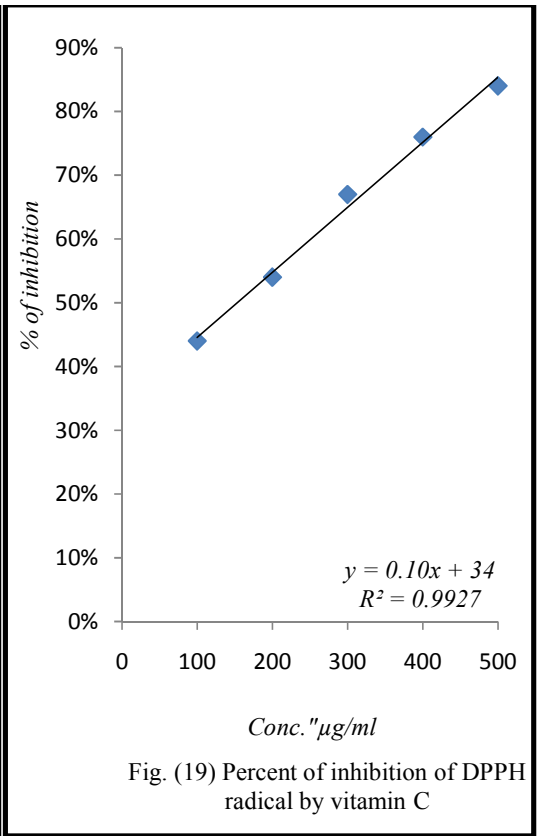


Fig. (19) Percent of inhibition of DPPH radical by vitamin C

Table (5): Phytochemical screening of *Ecballium elaterium* "fruit juice" and its absorbance.

Ecballium elaterium	Total phenolic content	Total flavonoid content	Reducing power	DPPH˙ radical
100 µl	0.525	0.0845	0.503	1.778
200 µl	0.725	0.135	0.875	1.478
300 µl	0.922	0.191	1.293	1.121
400 µl	1.154	0.223	1.563	0.447
500 µl	1.361	0.266	2.039	0.427

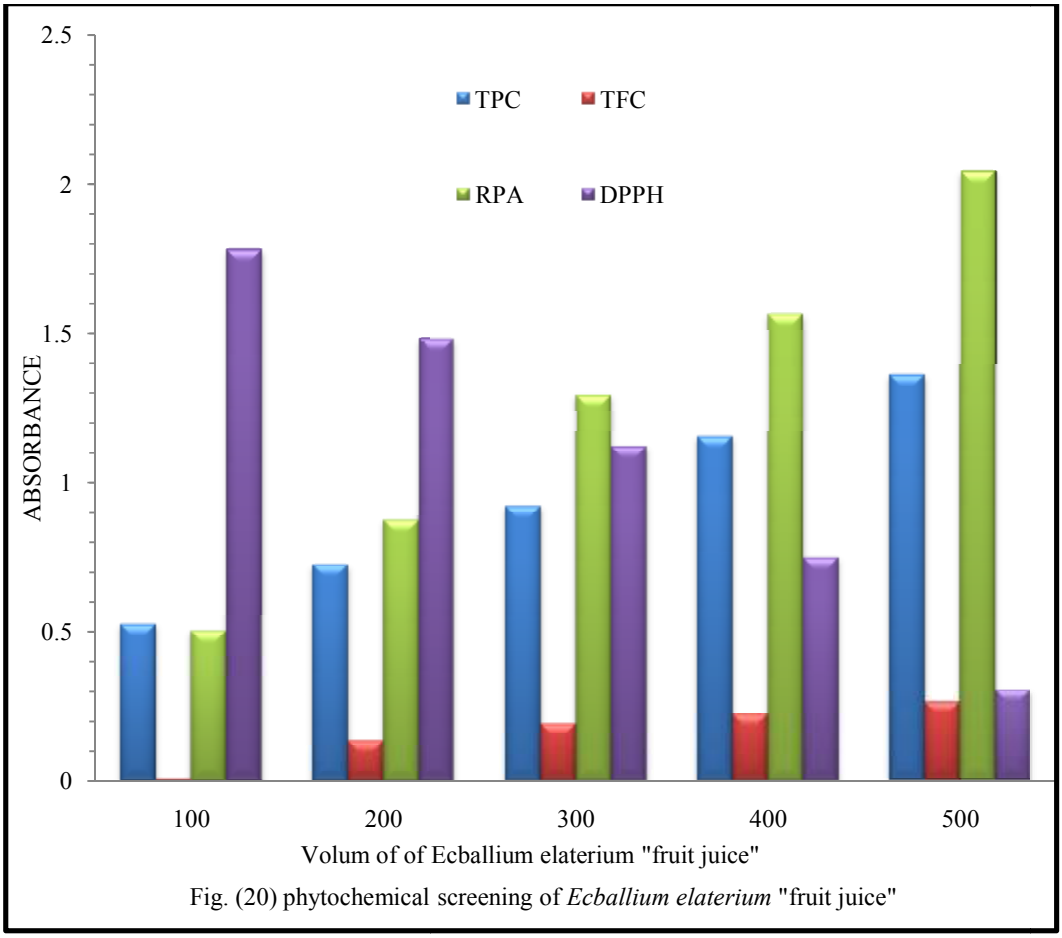


Fig. (20) phytochemical screening of *Ecballium elaterium* "fruit juice"

2. Hepatic marker enzymes in prophylactic group:

2.1. Effect of different treatments on serum ALT, AST, LDH, G-GT and ALP

The effects of acetaminophen on the serum enzymes ALT, AST, LDH, G-GT and ALP, illustrated in tables (6, 7, 8, 9 and 13), and figs. (21, 22, 23, 24, and 28), oral administration of acetaminophen significantly increased the activities of these enzymes by 332.68%, 166.80%, 204.29%, 258.60% and 70% respectively.

Pretreatment the rats with *Ecballium elaterium* "fruit juice" at 100 µl ameliorated these increases by 61.53%, 51.28%, 56.46%, 62.21% and 35.68% in ALT, AST, LDH, G-GT and ALP, respectively, when compared with positive group.

2.2. Effect of different treatments on serum proteins

There is significant increase in total bilirubin in table (12) and fig. (27) by 340%, the level of total protein and albumin significantly decreased by 49.44%, 66.38% respectively as illustrated in tables (10, 11) and figs. (25, 26).

There is decrease in total bilirubin levels by 40.88%, but the level of total protein and albumin increased by 61.67%, 135.78% after treatment with *Ecballium elaterium* "fruit juice" at 100 µl when compared with the acetaminophen treated group.

3. Renal function test in prophylactic group:

3.1. Effect of different treatments on serum creatinine and blood urea.

Serum creatinine and blood urea significantly increased after acetaminophen treatment by 334.6%, and 113.9% respectively, when compared with the control where noted in tables (14, 15) and figs. (29, 30). Serum creatinine and blood urea decreased by 62.8%, and 39.3% respectively when compared with the acetaminophen treated group.

Restoration of hepatic marker enzymes was noticed in the *Ecballium elaterium* "fruit juice" at 100 µl than the vitamin C at 300 mg/kg when compared with acetaminophen treated rats.

4. Antioxidant enzymes in prophylactic group:

After the exposure of rats to acetaminophen only a significant decrease in the activities of the antioxidant enzymes GR, GPx, CAT, and SOD, in comparison to the control group by 55.2%, 53.4%, 51.1%, and 60.7% respectively in tables (16, 17, 18, and 19) and figs. (31, 32, 33, and 34), but the MDA level shows significant increase by 153.5% table (20) and fig. (35).

Pretreatment of the rats with *Ecballium elaterium* "fruit juice" at (100µl) increase the activity of these enzymes GR, GPx, CAT, and SOD by 89.5%, 72.2%,

77.3%, and 111.5%, respectively and significant decrease in MDA by 44.3% when compared with the acetaminophen treated group as illustrated in tables (16, 17, 18, 19, and 20) and figs. (31, 32, 33, 34 and 35) respectively.

Table (6): Prophylactic effect of different treatments on S. ALT (U/L ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment	After treatment	% change
Control	41.34 ±7.33	43.45±3.72 †	5.10 ↑
Positive control	43.18±4.18	188.31±5.43 a,***	336.10 ↑
Ecballium elaterium fruit juice "100 µl"	44.31 ±2.99	72.43±4.66 b, ***	63.46 ↑
Vitamin C (300 mg/kg.b.w)	43.22 ±4.01	88.43±5.72 b, ***	104.6↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

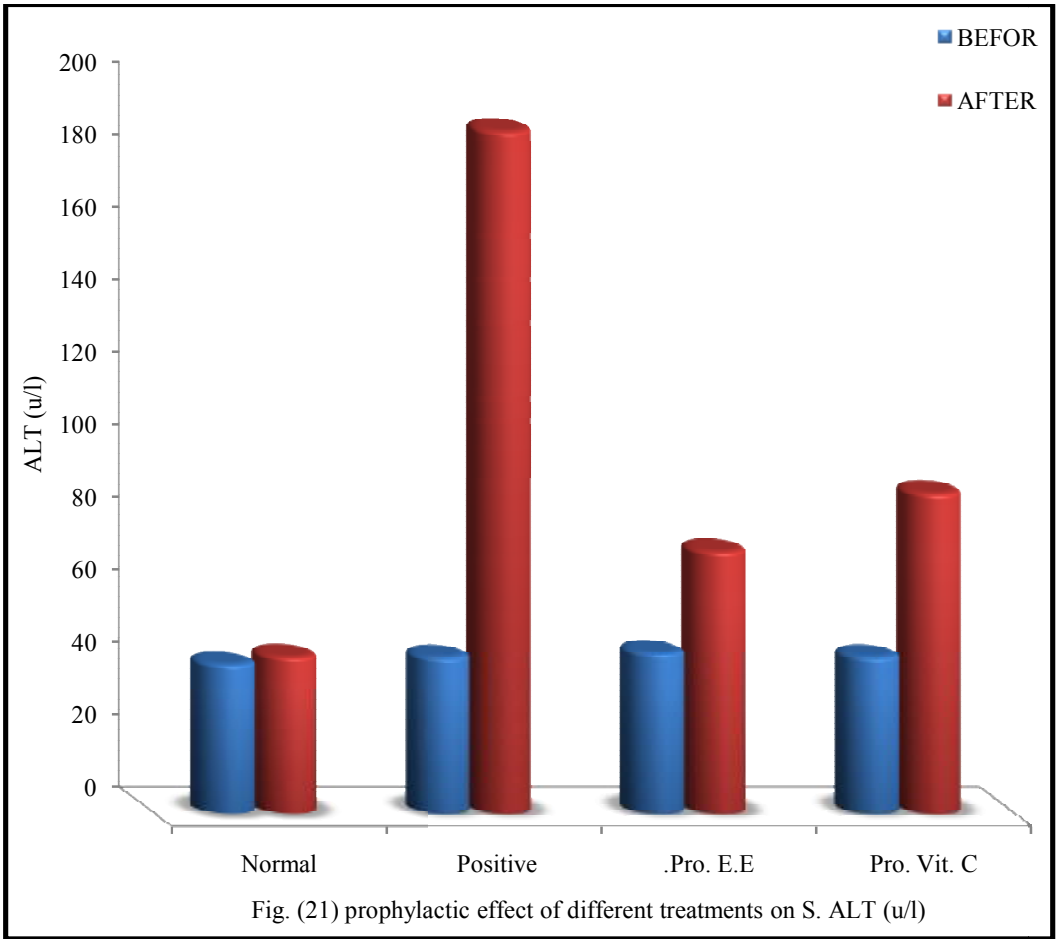


Fig. (21) prophylactic effect of different treatments on S. ALT (u/l)

Table (7): Prophylactic effect of different treatments on S. AST (U/L ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment	After treatment	% change
Control	93.41±8.01	96.37±4.22 †	3.17↑
Positive control	90.76±7.43	257.12±8.57 a, ***	183.29↑
Ecballium elaterium fruit juice "100 µl"	93.65±3.89	125.26±4.32 b, ***	33.75 ↑
Vitamin C (300 mg/kg.b.w)	91.22±5.43	138.65±5.78 b, ***	51.99 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

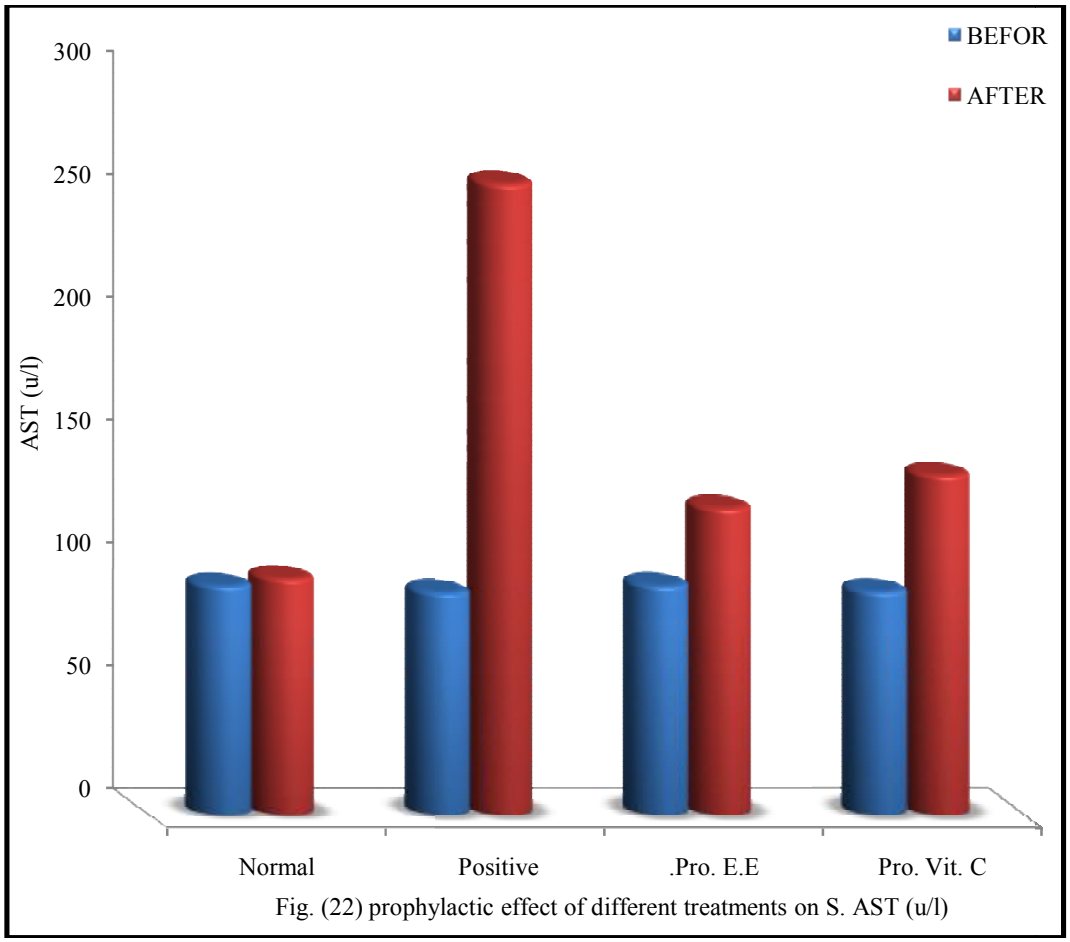


Fig. (22) prophylactic effect of different treatments on S. AST (u/l)

Table (8): Prophylactic effect of different treatments on S. LDH (U/L± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment	After Treatment	% change
Control	38.97±2.9	40.54±1.82 †	4.02 ↑
Positive control	41.21 ±2.5	123.36±4.52 a, ***	199.34 ↑
Ecballium elaterium fruit juice "100 µl"	41.94±2.1	53.71±3.22 b, ***	28.06 ↑
Vitamin C (300 mg/kg.b.w)	40.74±3.2	48.87±5.01 b, ***	19.95 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

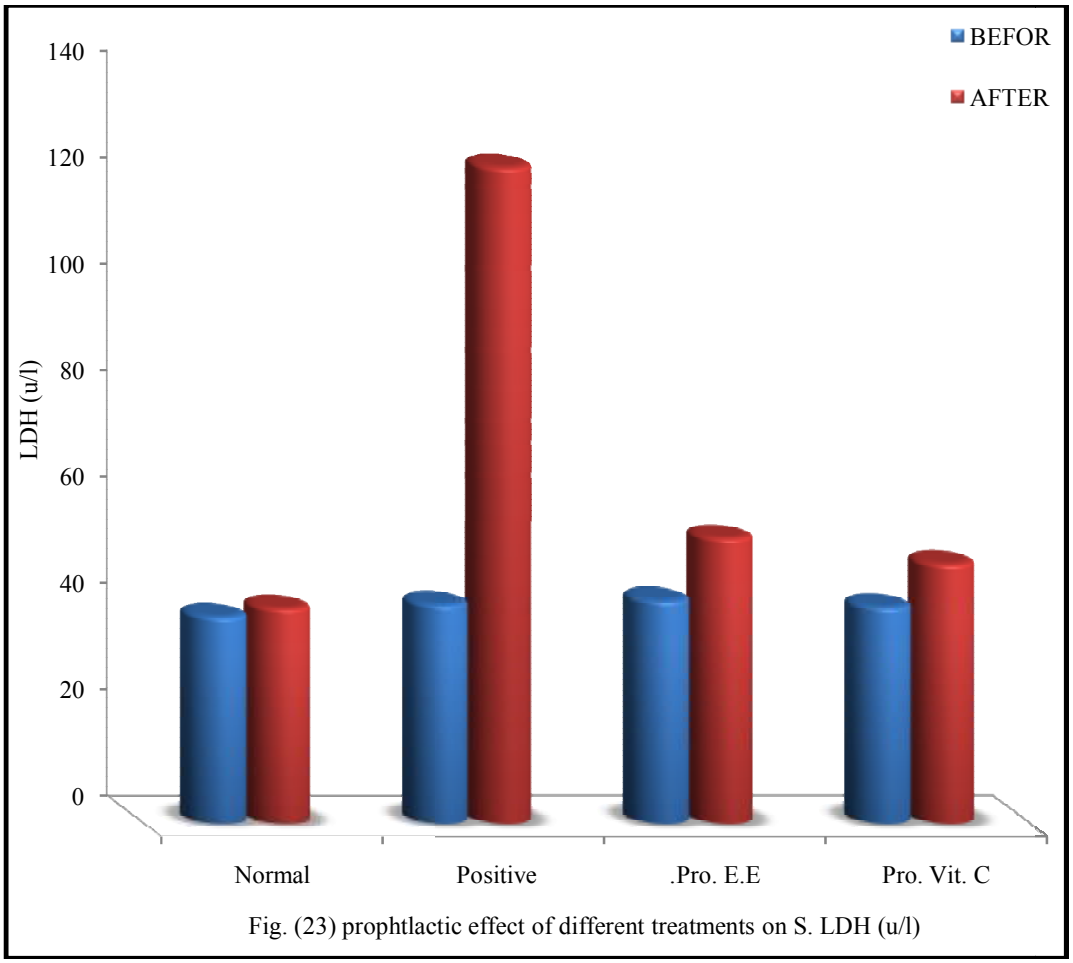


Fig. (23) prophtlactic effect of different treatments on S. LDH (u/l)

Table (9): Prophylactic effect of different treatments on S. G-GT (U/L ± S.D) and % change from the corresponding control during the induction of hypercholesterolemia for 3 weeks in male albino rats.

Animal groups	Before treatment	After Treatment	% change
Control	3.53±0.82	3.72±1.61[†]	5.38 ↑
Positive control	3.74±0.71	13.34±2.31 [a, ***]	256.68 ↑
Ecballium elaterium fruit juice "100 µl"	3.33±0.79	5.04±0.57 [b, ***]	51.53 ↑
Vitamin C (300 mg/kg.b.w)	3.51±0.65	5.85±0.97 [b, ***]	66.66 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with acetaminophen positive group.

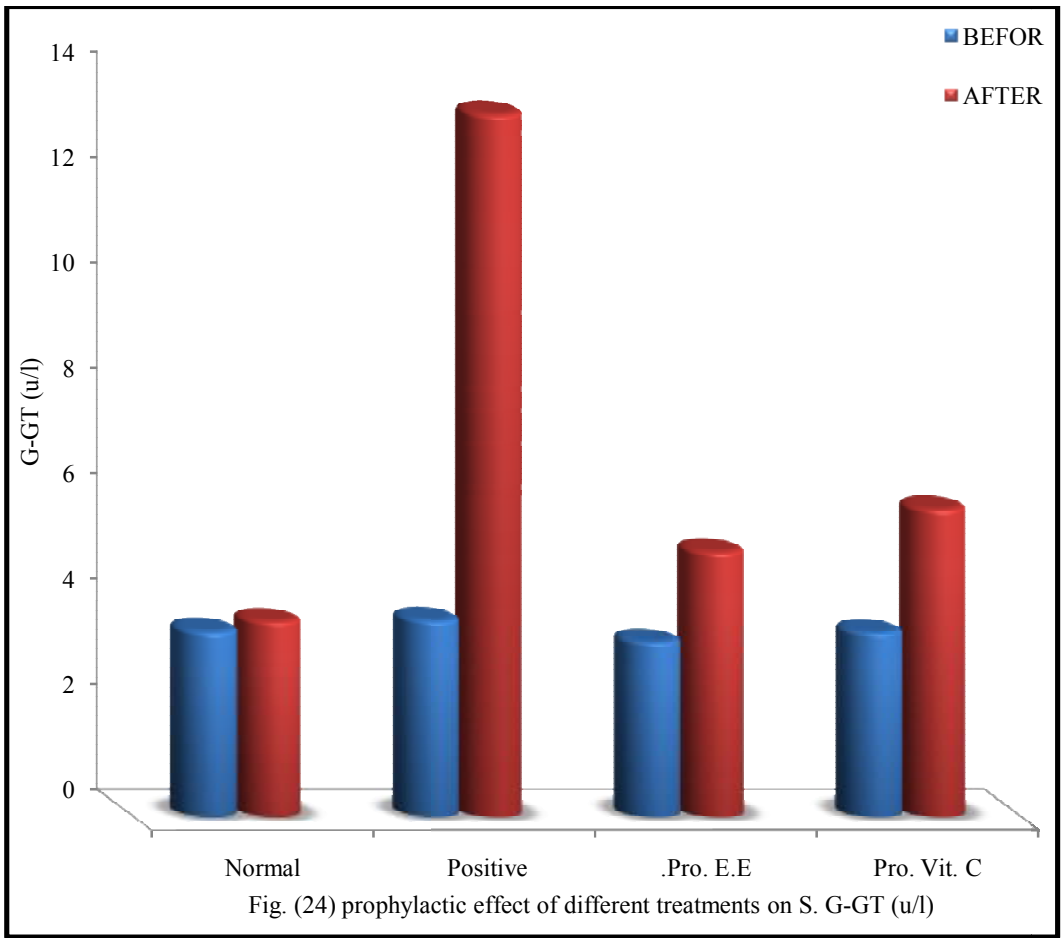


Fig. (24) prophylactic effect of different treatments on S. G-GT (u/l)

Table (10): Prophylactic effect of different treatments on S. total protein (g/dl ± S.D) and %change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment	After treatment	% change
Control	8.41±0.21	8.98±0.80 †	6.77 ↑
Positive control	9.12±0.49	4.54±0.23 a, ***	50.21 ↓
Ecballium elaterium fruit juice "100 µl"	8.97±0.38	7.34±0.66 b, ***	22.20 ↓
Vitamin C (300 mg/kg.b.w)	8.93±0.47	6.84±0.54 b, ***	23.40 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

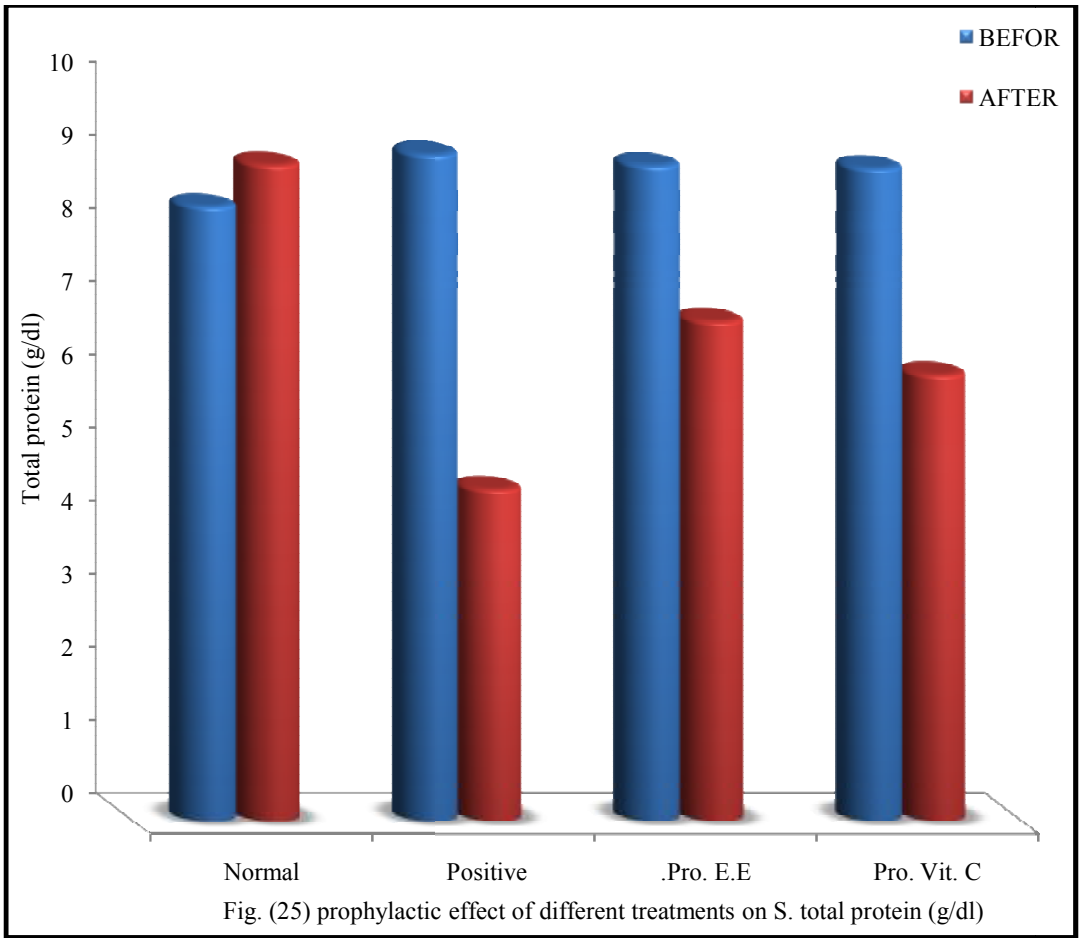


Fig. (25) prophylactic effect of different treatments on S. total protein (g/dl)

Table (11): Prophylactic effect of different treatments on S. albumin (g/dl ±S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment	After treatment	% change
Control	8.83±0.27	9.31±0.53†	5.43 ↑
Positive control	8.51±0.36	3.13±0.28 a, ***	63.21 ↓
Ecballium elaterium fruit juice "100 µl"	9.31±0.17	7.38±0.11 b, **	19.16 ↓
Vitamin C (300 mg/kg.b.w)	8.77±0.51	6.93±0.47 b, **	20.98 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

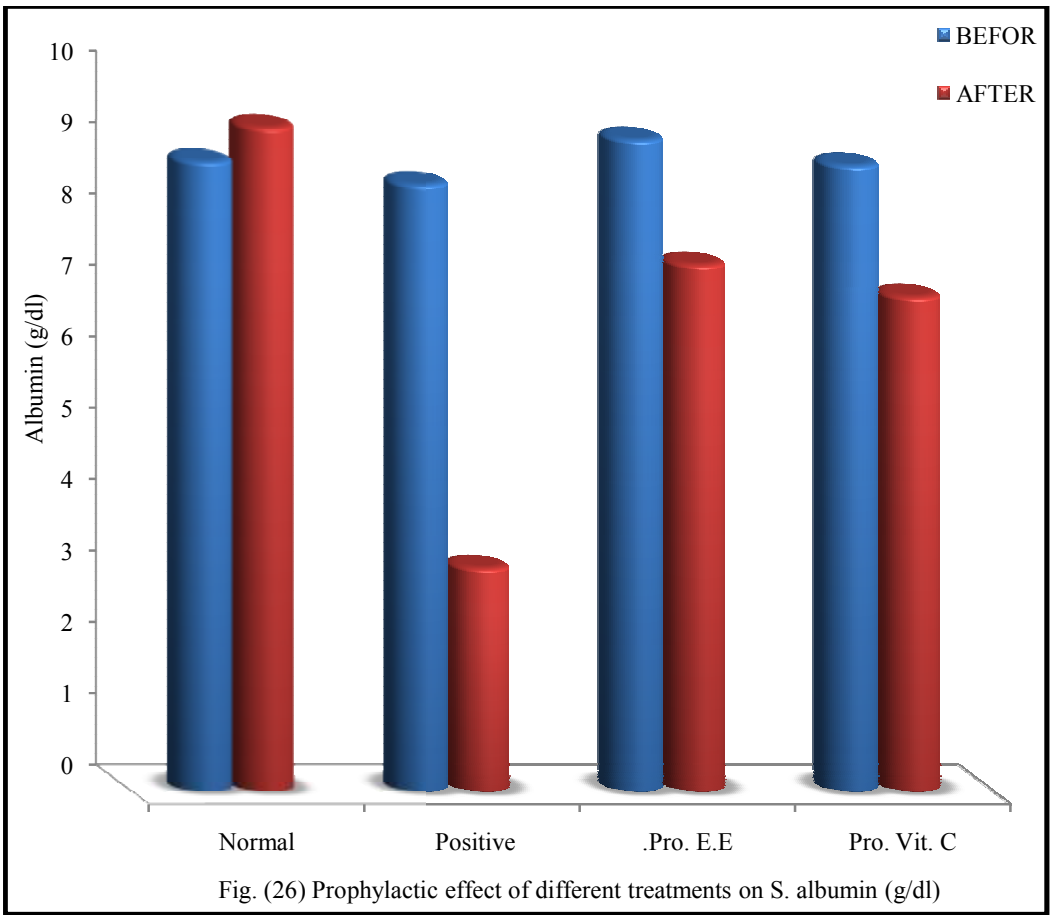


Fig. (26) Prophylactic effect of different treatments on S. albumin (g/dl)

Table (12): Prophylactic effect of different treatments on total bilirubin (mg/dl± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment	After Treatment	% change
Control	0.42±0.21	0.45±0.14[†]	7.14 ↑
Positive control	0.45±0.27	1.98±0.36 [a, ***]	340 ↑
Ecballium elaterium fruit juice "100 µl"	0.43±0.57	0.66±0.23 [b, ***]	53.48 ↑
Vitamin C (300 mg/kg.b.w)	0.42±0.017	0.71±0.35 [b, ***]	69 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with acetaminophen positive group.

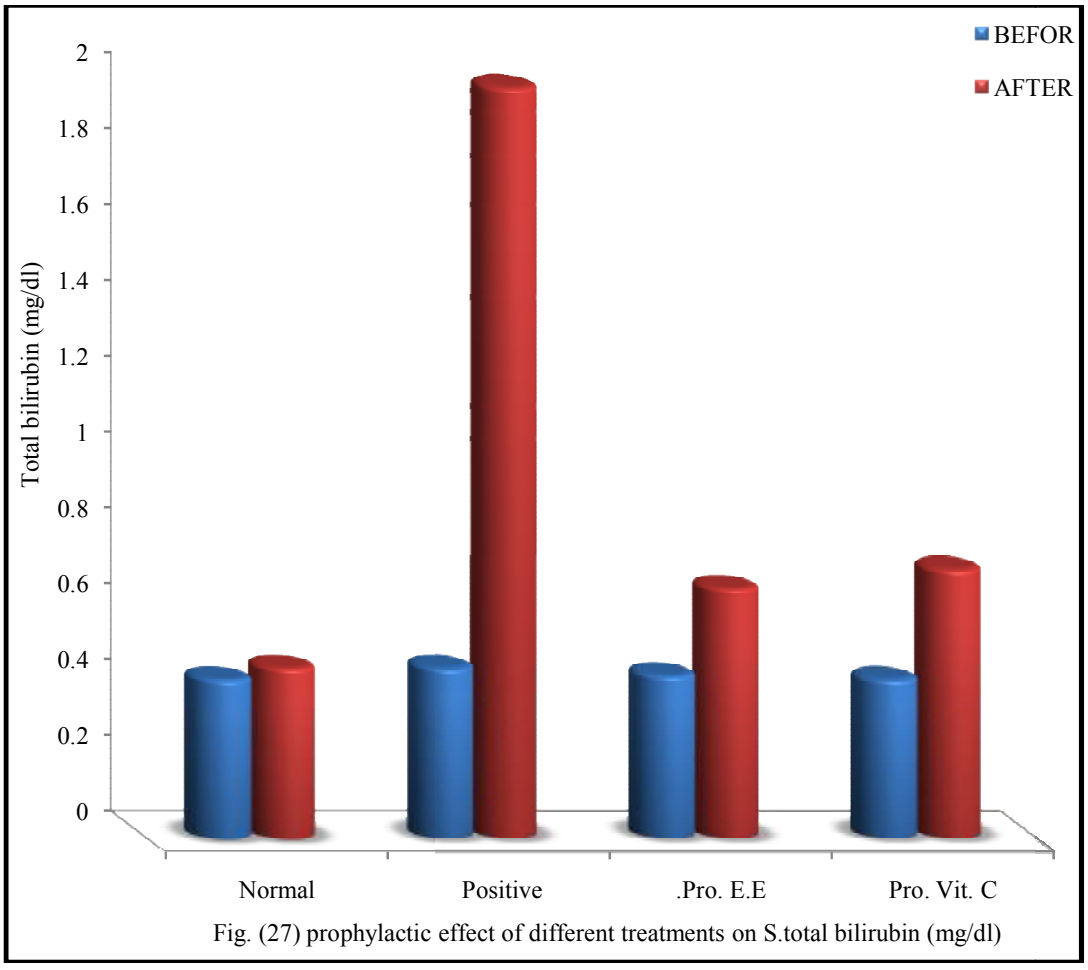


Fig. (27) prophylactic effect of different treatments on S.total bilirubin (mg/dl)

Table (13): Prophylactic effect of different treatments on S. ALP (U/L ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment	After Treatment	% change
Control	147±2.0	150±4.83 †	2.04 ↑
Positive control	149±6.99	255±2.53 a, ***	71.14 ↑
Ecballium elaterium fruit juice "100 µl"	148 ±4.36	164±4.75 a, ***	10.8 ↑
Vitamin C (300 mg/kg.b.w)	152±7.01	171±3.93 a, ***	12.5 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

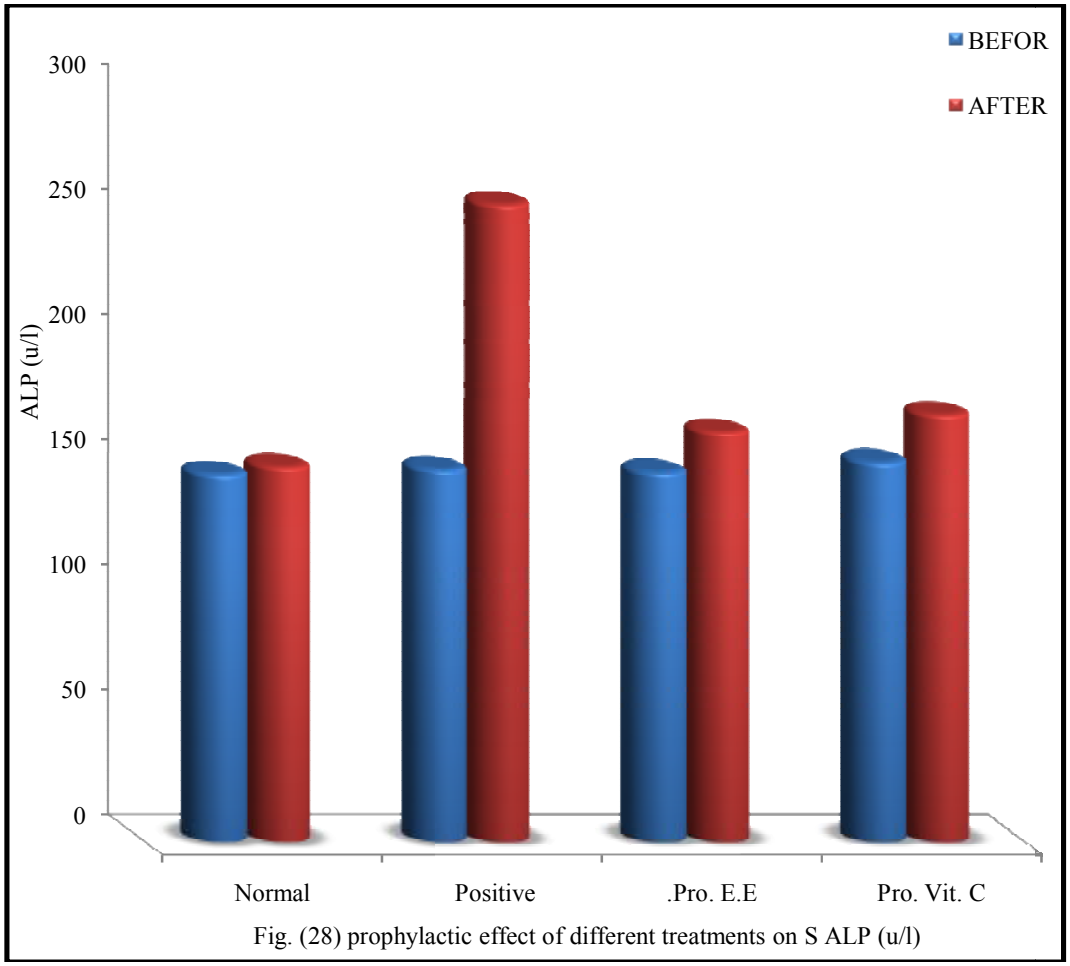


Fig. (28) prophylactic effect of different treatments on S ALP (u/l)

Table (14): Prophylactic effect of different treatments on S. creatinine (mg/dL ± S. D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment	After treatment	% change
Control	0.24 ± 0.02	0.26 ± 0.14 †	8.33 ↑
Positive control	0.27 ± 0.05	1.13 ± 0.17 a, ***	318.5 ↑
Ecballium elaterium fruit juice "100 µl"	0.24 ± 0.01	0.42 ± 0.12 b, ***	75.83 ↑
Vitamin C (300 mg/kg.b.w)	0.25 ± 0.02	0.35± 0.14 b, ***	40 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group ; b compared with acetaminophen positive group.

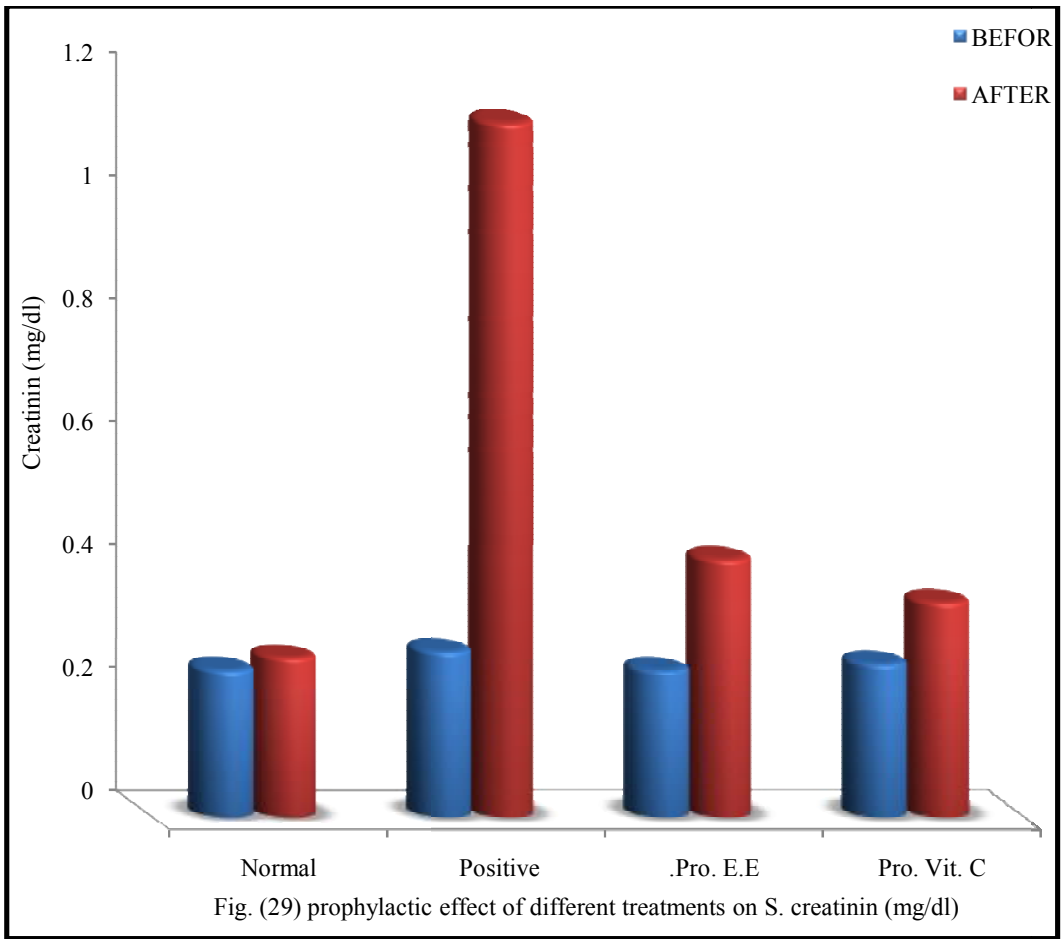


Fig. (29) prophylactic effect of different treatments on S. creatinin (mg/dl)

Table (15): Prophylactic effect of different treatments on blood urea (mg/dL ± S. D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before Treatment	After treatment	% change
Control	21.42 ± 1.12	22.13 ± 1.99 †	3.31 ↑
Positive control	21.45 ± 1.11	47.34± 2.73 a, ***	120.7 ↑
Ecballium elaterium fruit juice "100 µl"	20.23 ±1.43	28.71±2.01 b, **	41.92 ↑
Vitamin C (300 mg/kg.b.w)	21.18 ± 1.99	25.31 ± 2.01 b, **	19.5 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

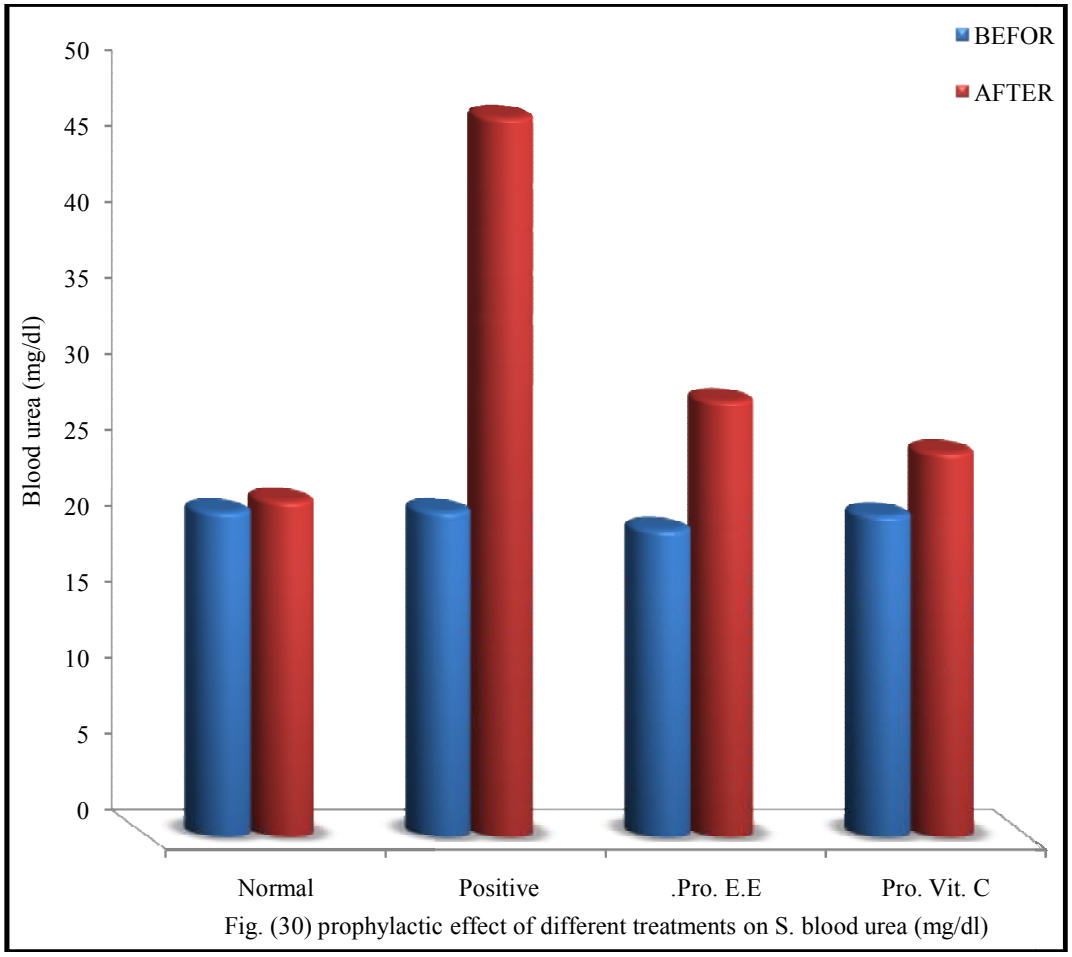


Fig. (30) prophylactic effect of different treatments on S. blood urea (mg/dl)

Table (16): Prophylactic effect of different treatments on plasma GR (u/l ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment	After treatment	% change
Control	25.28±0.02	26.14±0.05 †	3.40 ↑
Positive control	26.67±0.01	11.71±0.01 a, ***	56.09 ↓
Ecballium elaterium fruit juice "100 µl"	25.76±1.69	22.19±0.02 b, ***	13.85 ↓
Vitamin C (300 mg/kg.b.w)	26.23±0.04	20.37±0.02 b, ***	22.34 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

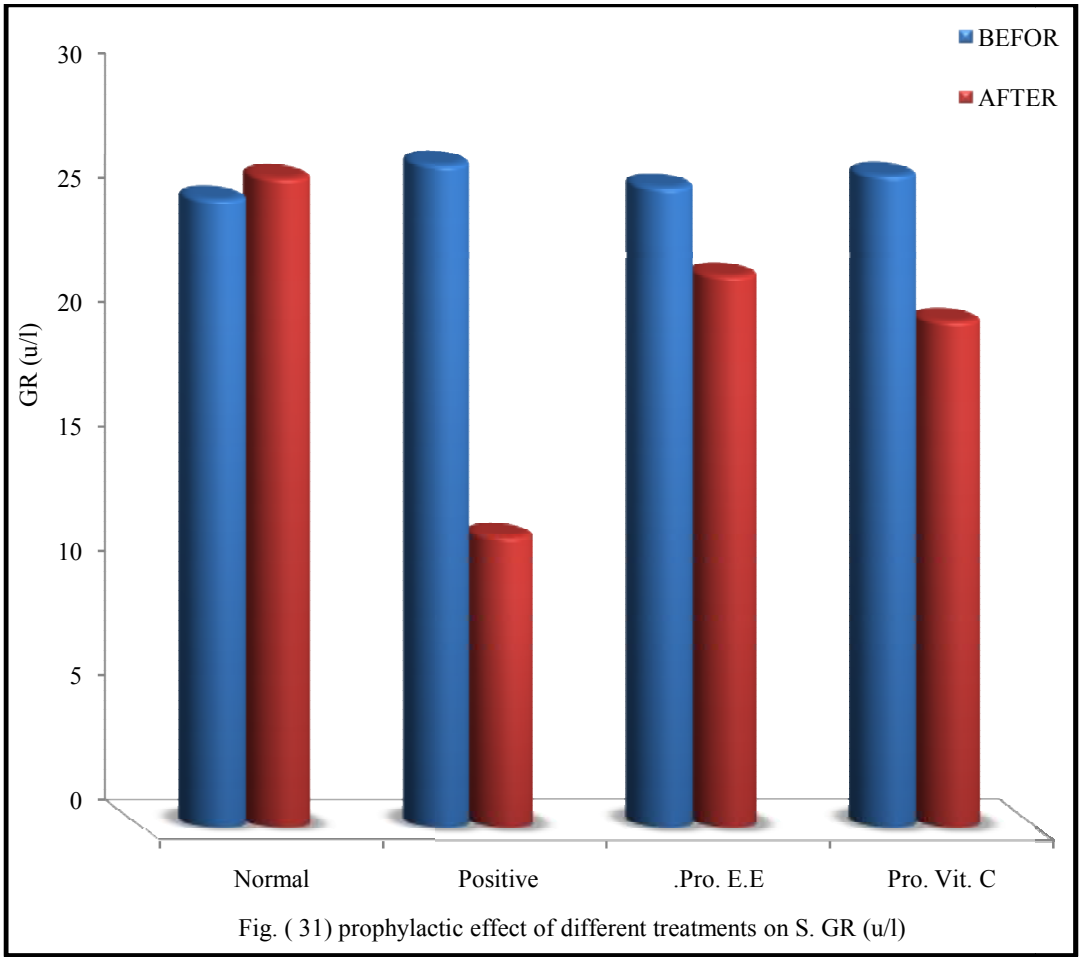


Fig. (31) prophylactic effect of different treatments on S. GR (u/l)

Table (17): Prophylactic effect of different treatments on plasma GP_X (mu/ml ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment	After treatment	% change
Control	30.67±1.15	30.72±0.39 †	0.16 ↑
Positive control	31.23±2.16	14.31 ± 0.03 [a, **]	54.17 ↓
Ecballium elaterium fruit juice "100 µl"	30.71±1.14	24.65±1.21 [b, *]	19.73 ↓
Vitamin C (300 mg/kg.b.w)	33.17±2.44	21.23±2.14 [b, *]	36 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group ; [b] compared with acetaminophen positive group.

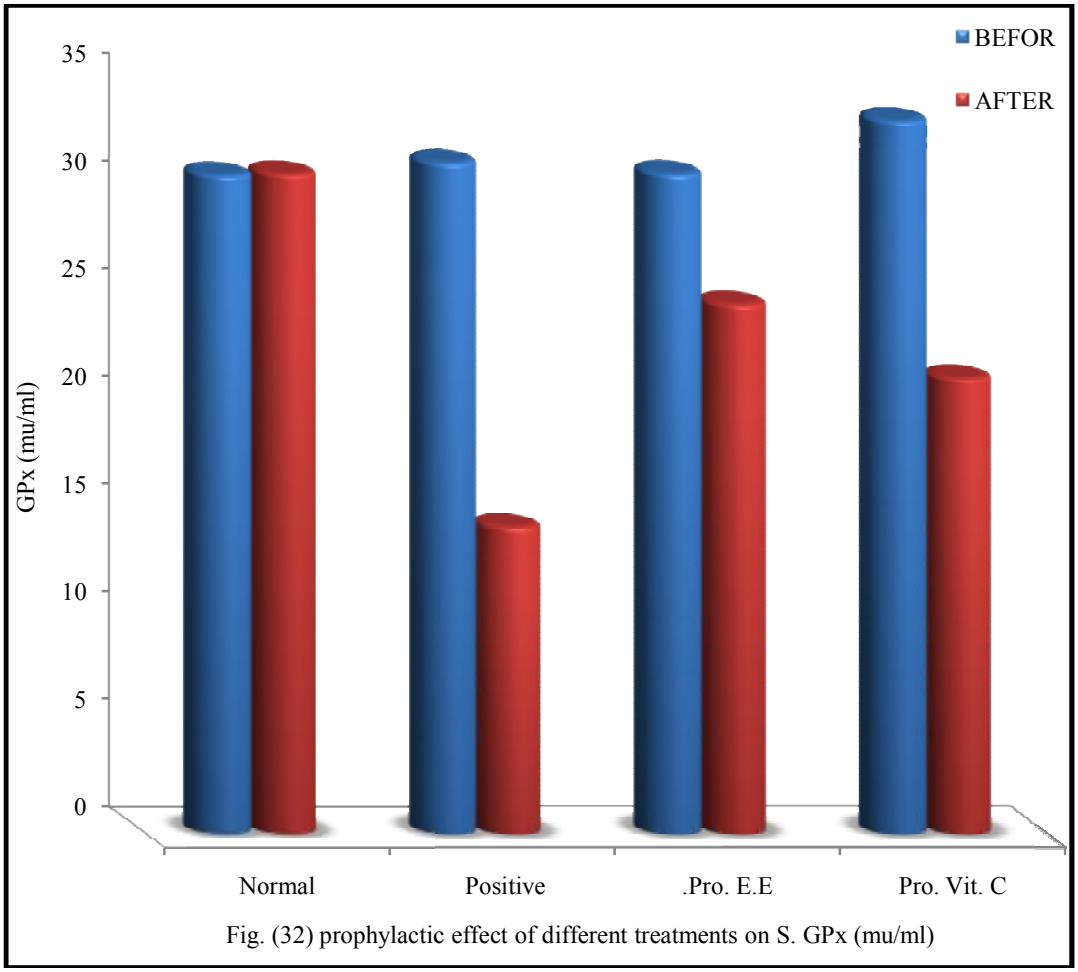


Fig. (32) prophylactic effect of different treatments on S. GPx (mu/ml)

Table (18): Prophylactic effect of different treatments on plasma CAT (u/l ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment	After treatment	% change
Control	45.38±3.29	46.45±3.99 †	2.36 ↑
Positive control	46.31±4.55	22.71±3.32 a, **	50.96 ↓
Ecballium elaterium fruit juice "100 µl"	47.66±3.37	40.26±5.01 b, **	15.52 ↓
Vitamin C (300 mg/kg.b.w)	46.98±2.44	38.11±4.77 b, **	18.88 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

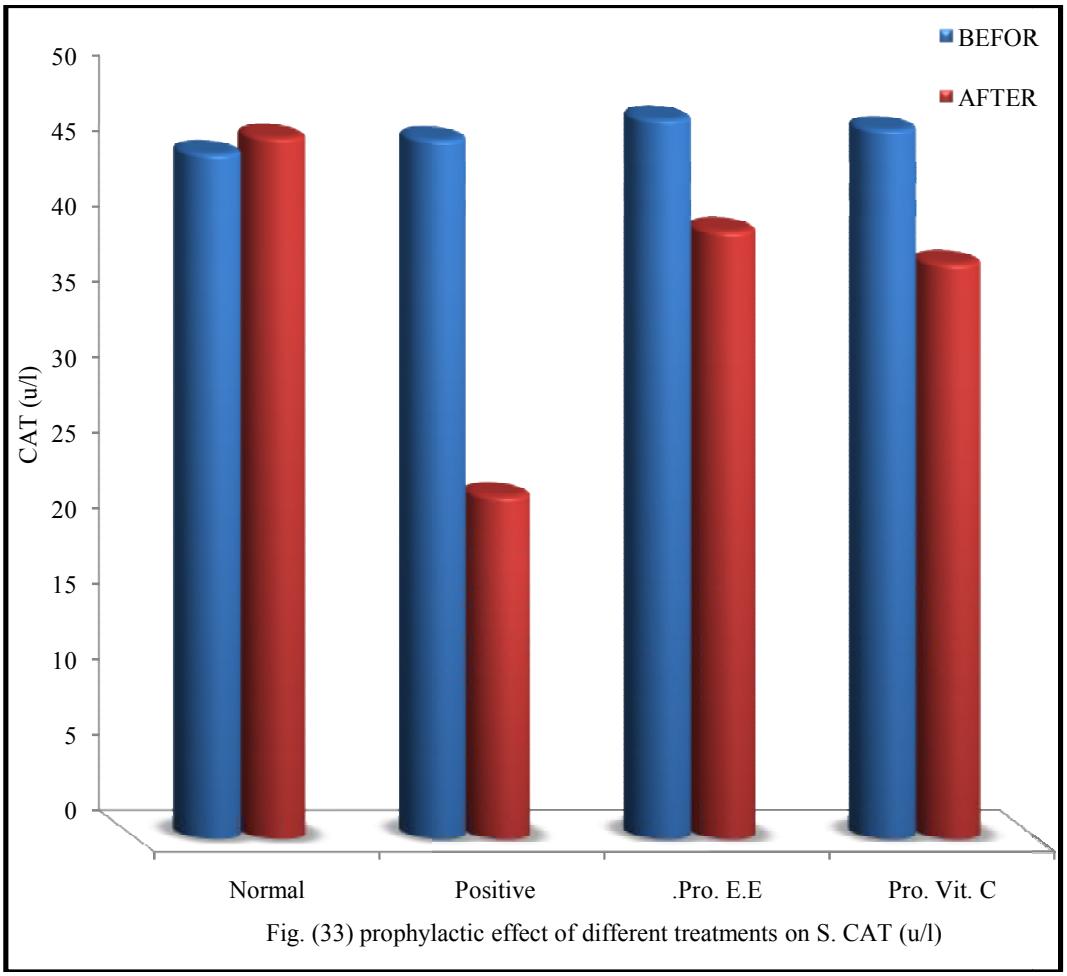


Fig. (33) prophylactic effect of different treatments on S. CAT (u/l)

Table (19): Prophylactic effect of different treatments on plasma SOD (u/mol ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment	After treatment	% change
Control	12.31±2.97	13.53±1.93 †	9.91 ↑
Positive control	13.71±1.84	5.31±1.57 a, ***	61.26 ↓
Ecballium elaterium fruit juice "100 µl"	14.41±2.01	11.23±2.21 b, ***	22.06 ↓
Vitamin C (300 mg/kg.b.w)	12.93±2.13	9.81±2.31 b, **	24.13 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

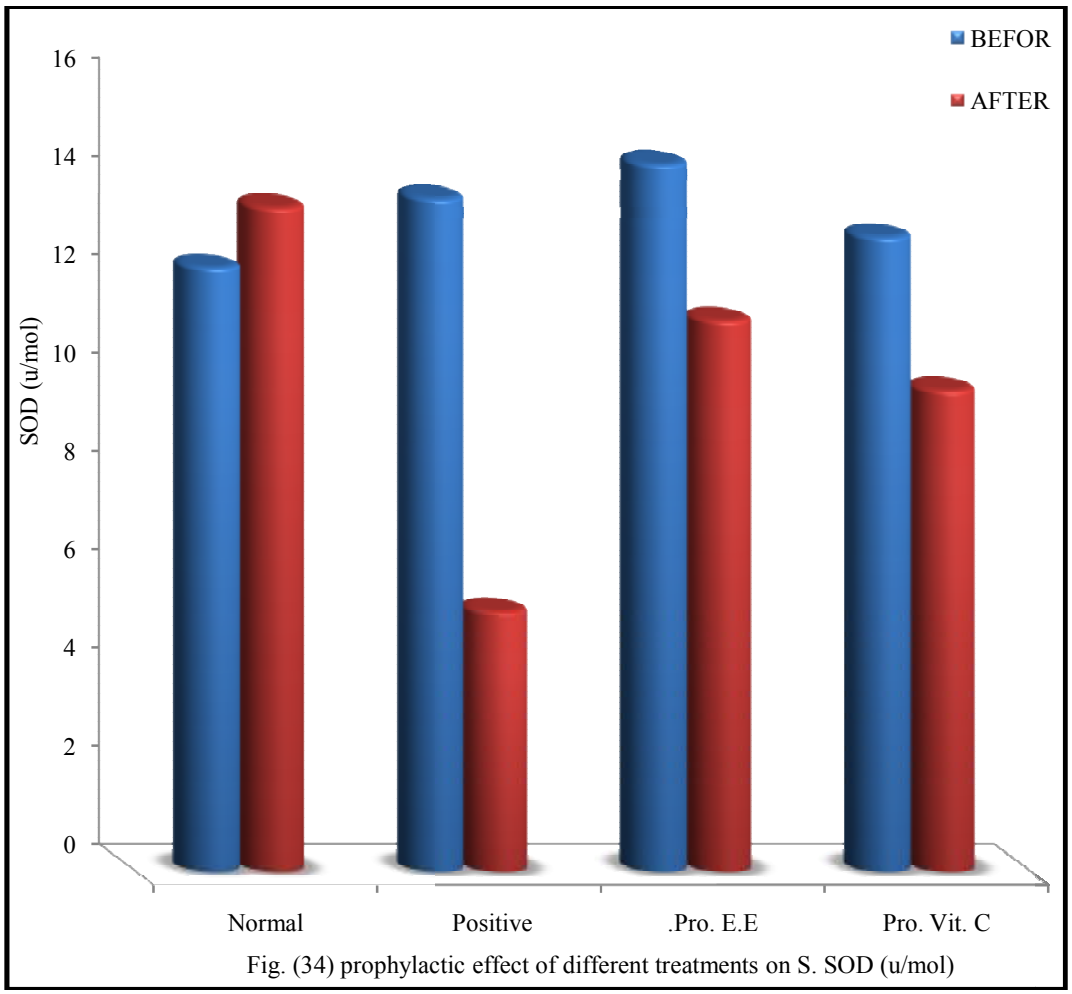


Fig. (34) prophylactic effect of different treatments on S. SOD (u/mol)

Table (20): Prophylactic effect of different treatments on plasma MDA (nmol/ml ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment	After treatment	% change
Control	18.92±3.22	19.35±3.66 †	2.27 ↑
Positive control	18.37±3.21	49.05±2.31 a, ***	167 ↑
Ecballium elaterium fruit juice "100 µl"	17.20±4.23	27.30±3.09 b, **	58.72 ↑
Vitamin C (300 mg/kg.b.w)	18.20±3.78	22.11±4.05 b, **	21.48 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

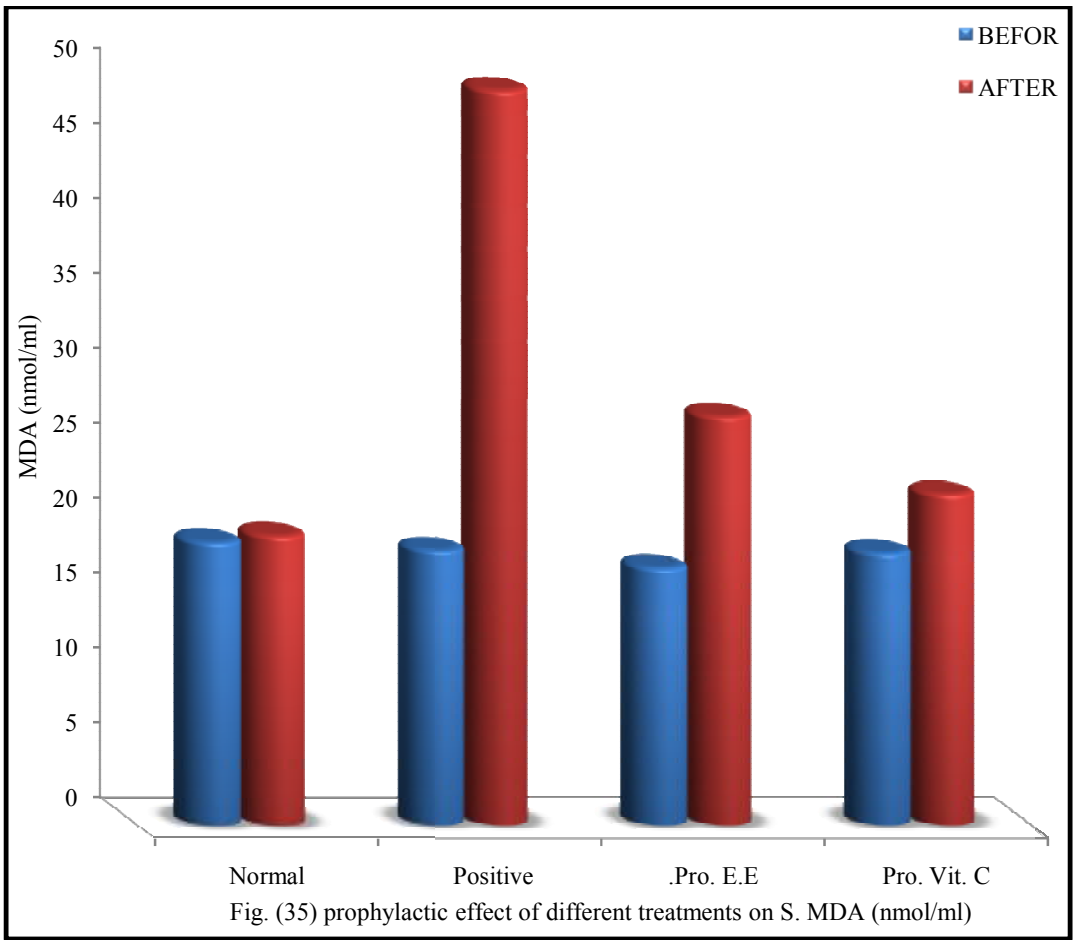


Fig. (35) prophylactic effect of different treatments on S. MDA (nmol/ml)

4. Histopathology of the liver:

The results of light microscopy examination of the transverse section of control, acetaminophen -treated and treated rats liver are shown in figs. (36, 37, 38, and 39).

In fig. (36) the liver cells of rat in the control group, from that image it can be observed that the normal liver architecture of hepatocytes, the cells are intact and most importantly, the portal vein has a regular shape, overall, a healthy set of cells can be observed.

In the rats treated with acetaminophen alone, induced marked histopathological lesion which was characterized by diffuse ballooning degeneration, dusty and cloudy swelling, fatty degeneration, hepatocellular necrosis, and irregular appearance due to the damage of cells were seen in fig. (37). when compared to the hepatocytes architecture of normal liver fig. (36).

The hepatocytes distortion was ameliorated where near normal appearance of hepatocytes in *Ecballium elaterium* "fruit juice" at 100 µl in pretreated livers, also at the 300 mg/kg as illustrated in (figs. 38, 39) The most significant ameliorating effect was recorded for 100 µl of *Ecballium elaterium* "fruit juice" pretreated liver (fig. 38)

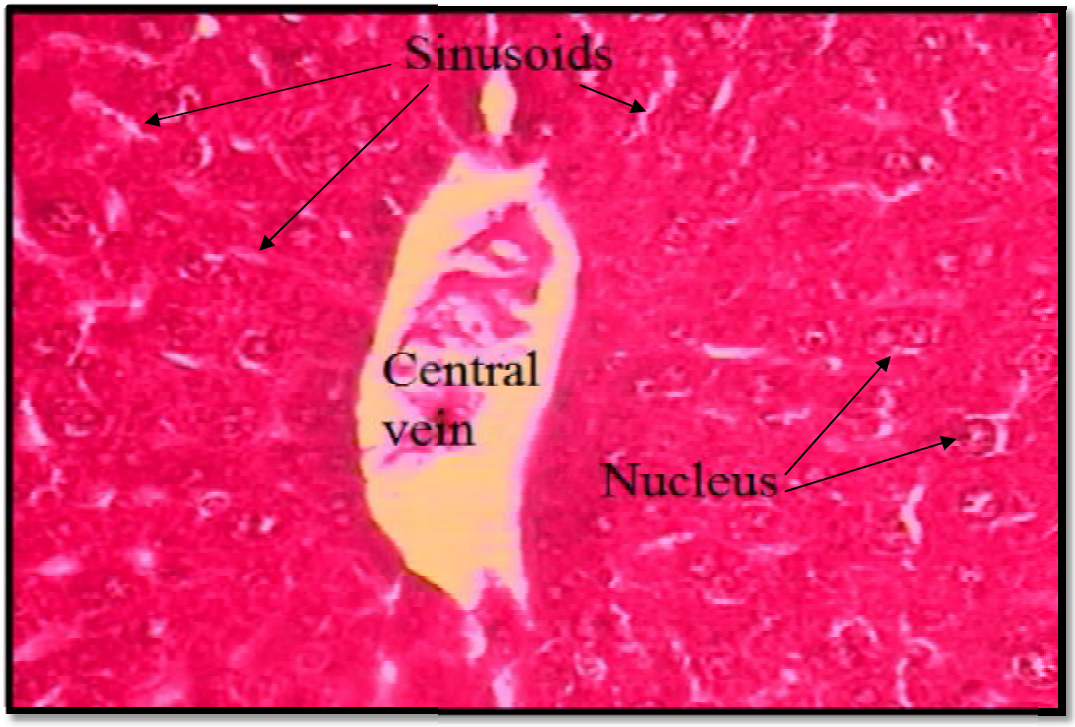


Fig. (36) light microscopy of liver cell of normal rat.

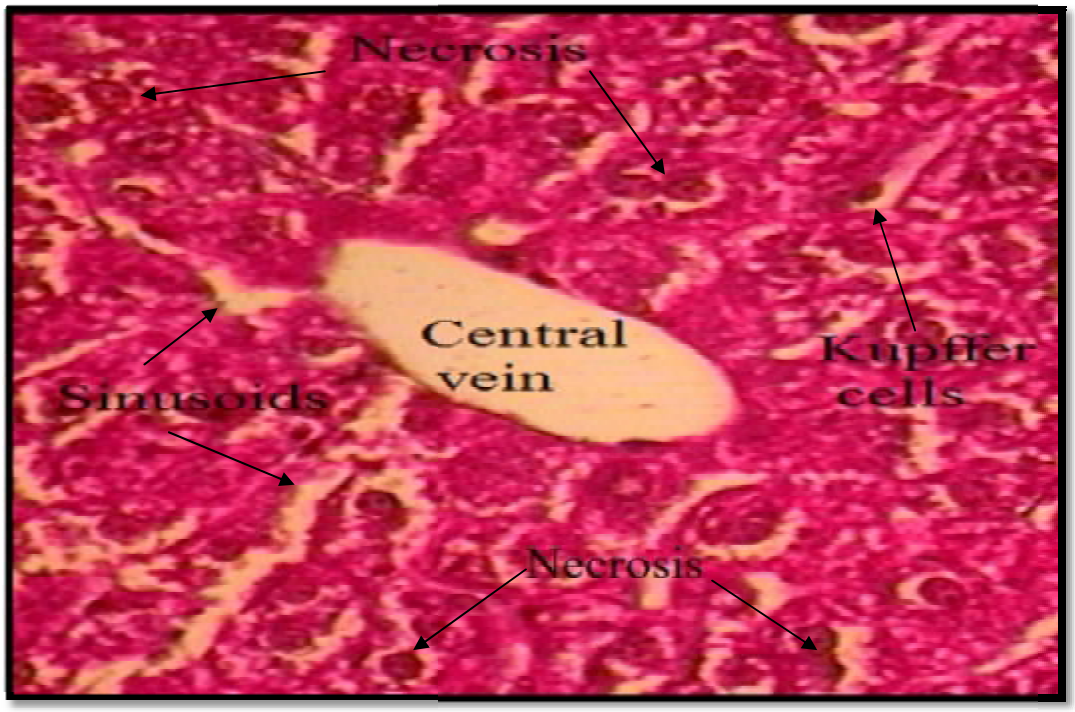


Fig. (37) Light microscopy of liver cell of positive rat treated by 400mg/kg of acetaminophen.

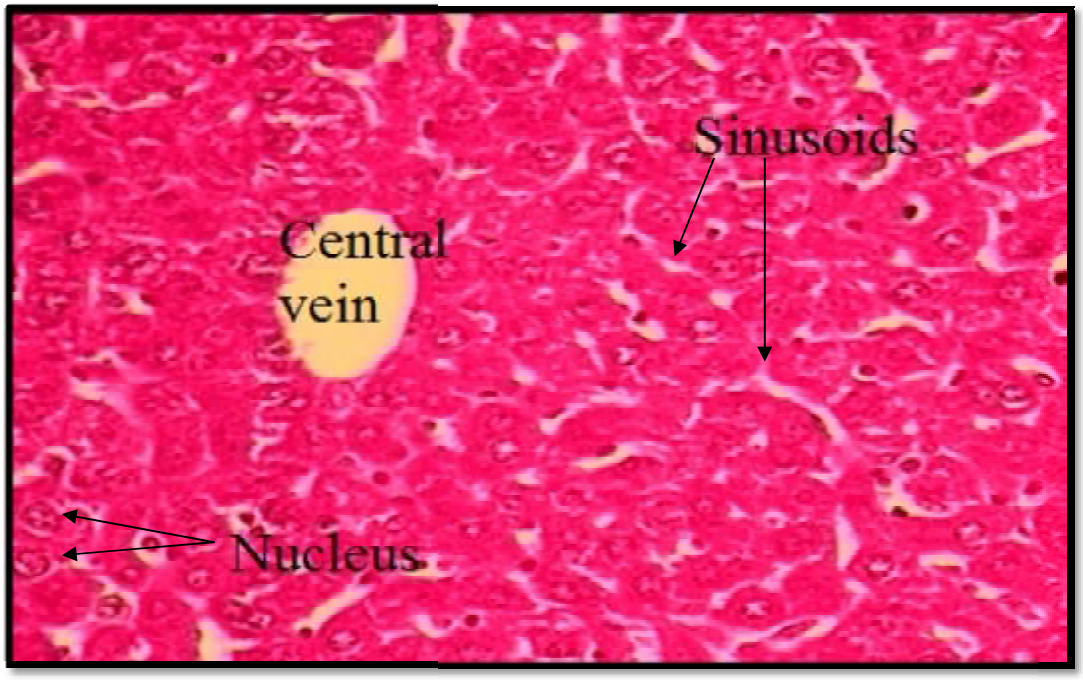


Fig. (38) Light microphotographs of liver cell treated by *Ecballium elaterium* "fruit juice" at 100 µl

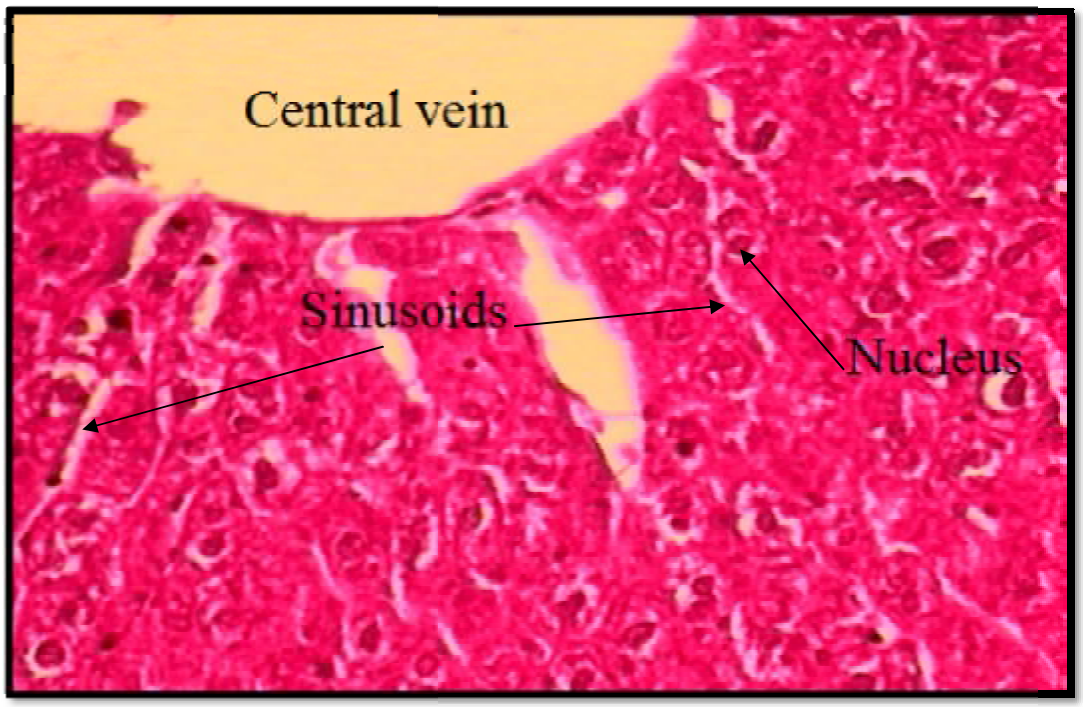


Fig. (39) Light microphotographs of liver cell treated by vitamin C at 300 mg/kg

5. Induction of hepatotoxicity by acetaminophen.

Table (21): Arithmetic mean values ± S.D and % change from the corresponding control of different biochemical parameters before and after induction of hepatotoxicity for 3 weeks in male albino rats.

Parameter	Before induction of hepatotoxicity	After induction of hepatotoxicity	% change
S.ALT (u/ml)	43.57±2.13	193.61±6.22 ***	344.36 ↑
S.AST (u/ml)	93.76 ±5.42	255.76±5.17 ***	172.78 ↑
S. LDH (u/l)	42.46±3.81	127.14±4.76 ***	199.43 ↑
S. G-GT (u/l)	3.22±1.82	12.98±3.83 ***	303.10 ↑
S. Total Protein (g/dl)	9.00±1.65	4.15±1.27 ***	53.88 ↓
S. Albumin(g/dl)	8.75±0.57	3.22±0.33 ***	63.20 ↓
S. Total Bilirubin (mg/dl)	0.45±0.23	2.14±0.96 ***	375.55 ↑
S. ALP (u/l)	147.34±5.77	270.67±5.43 ***	83.70 ↑
S. Creatinine (mg/dl)	0.25±0.73	1.22±0.74 ***	388 ↑
S. Blood urea (mg/dl)	22.98±3.11	43.77±5.88 ***	90.47 ↑
P. GR (u/l)	28.97±2.69	8.97±2.11 ***	69.03 ↓
P. GPx (mu/ml)	32.74±3.27	12.02±1.93 ***	63.28 ↓
P. CAT (u/l)	47.93±5.12	21.28±3.74 ***	55.60 ↓
P. SOD (u/mol)	12.51±3.44	4.86±1.39 ***	61.15 ↓
S.MDA (nmol/ml)	21.20±2.93	49.04±2.89 ***	131.32 ↑

† Nonsignificant difference from the corresponding control at $P > 0.1$; * Significant difference at $P < 0.05$; ** highly sig. difference at $P < 0.01$; *** Very highly sig. difference at $P < 0.001$; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with acetaminophen positive group.

6. Hepatic marker enzymes in Curative group:

6.1. Effect of different treatments on serum ALT, AST, LDH, G-GT and ALP.

The effect of acetaminophen on the serum enzymes ALT, AST, LDH, G-GT and ALP. As illustrated in tables (22, 23, 24, 25 and 29), and figs. (40, 41, 42, 43, 47), oral administration of acetaminophen significantly increases the activity of these enzymes by 383.66%, 184.4%, 234.7%, 235.1% and 82.5% respectively.

Post treatment of the rats with *Ecballium elaterium* "fruit juice" at (100µl) ameliorated these increases by 49.76%, 31.40%, 47.46%, 41.1% and 27.58% in ALT, AST, LDH, G-GT and ALP, respectively.

6.2. Effect of different treatments on serum proteins.

The level of total protein and albumin tables (26, 27) and figs. (44, 45), significantly decreased by 58.04%, 57.25% respectively also there is significant increases in total bilirubin in table (28) and fig. (46) by 332.5% in acetaminophen treated group when compared with normal group.

The use of *Ecballium elaterium* "fruit juice" at 100 µl as a post treatment increase the level of total protein and albumin by 72.57%, 74.45% but there was decrease in the level of total bilirubin by 52.02% respectively, when compared with acetaminophen treated group.

7. Renal function test in Curative group:

7.1.Effect of different treatment on serum creatinine and blood urea.

Serum creatinine and blood urea significantly increased by 324.13%, and 116.8% respectively, in acetaminophen treated group where noted in tables (30, 31) and figs. (48, 49), when compared with the control.

When treated by 100 µl of *Ecballium elaterium* "fruit juice" post treatment the level of serum creatinine and blood urea decreased by 48.78%, 21.2% respectively when compared with the acetaminophen treated group.

Restoration of hepatic marker enzymes was noticed more in the *Ecballium elaterium* "fruit juice" at (100µl) than the vitamin C at (300 mg/ kg) and silymarin at 50 mg/kg when compared with acetaminophen treated group.

8. Antioxidant enzymes in curative group:

After the exposure of rats to acetaminophen only a significant decrease in the activities of GR, GPx, CAT, and SOD, in comparison to the control group by 63.8%, 66.9%, 62.2%, and 75.3% respectively tables (32, 33, 34, 35) and figs. (50, 51, 52, 53) the MDA level shows significant increase by 138% table (36) and fig. (54).

Post treatment of the rats with *Ecballium elaterium* "fruit juice" at (100µl) increase the activity of these enzymes GR, GPx, CAT, and SOD by 94.15%, 73.43%, 75.5%, and 90.3%, respectively and significant decrease in MDA by 29.39% when compared with the acetaminophen treated group.

Table (22): Curative effect of different treatments on S. ALT (U/L ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After Treatment	% change
Control	40.21± 4.85	41.38±5.43[†]	2.9 ↑
Positive control	193.34±12.0	200.14±16.0 [a, ***]	3.51 ↑
Ecballium elaterium fruit juice "100 µl"	197.23±9.01	100.55±11.12 [b, ***]	49 ↓
Vitamin C (300 mg/kg.b.w)	190.96±13.2	126.27±8.44 [b, ***]	33.9 ↓
Silymarin (50 mg/kg b.w.)	192.92±11.2	117.78±14.42 [b, ***]	39 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with acetaminophen positive group.

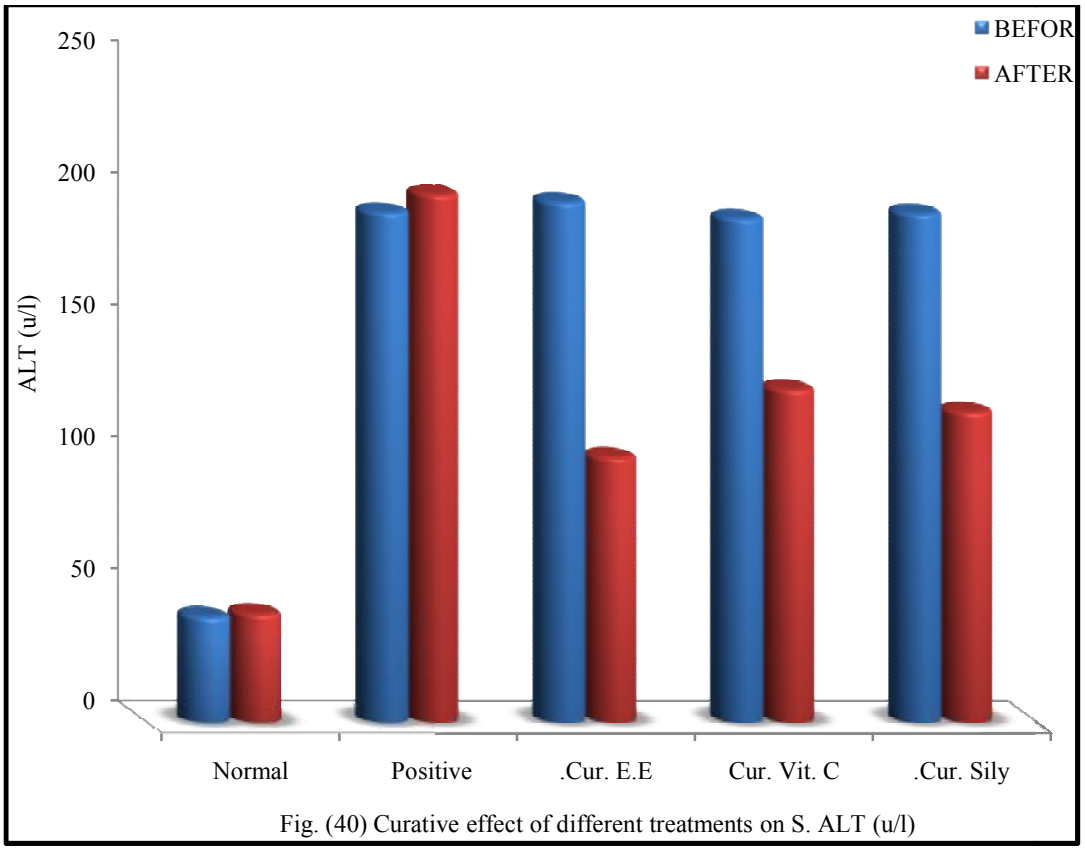


Fig. (40) Curative effect of different treatments on S. ALT (u/l)

Table (23): Curative effect of different treatments on S. AST (U/L ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After Treatment	% change
Control	89.79± 7.22	91.52±9.85 †	1.92 ↑
Positive control	265.67±22.3	260.32±17.3 a, ***	2.01 ↓
Ecballium elaterium fruit juice "100 µl"	252.28±10.7	178.57±11.2 b, ***	29.21 ↓
Vitamin C (300 mg/kg.b.w)	258.63±17.3	188.41±9.52 b, ***	27.15 ↓
Silymarin (50 mg/ kg b.w.)	255.47±9.37	194.61±7.45 b, ***	23.82 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

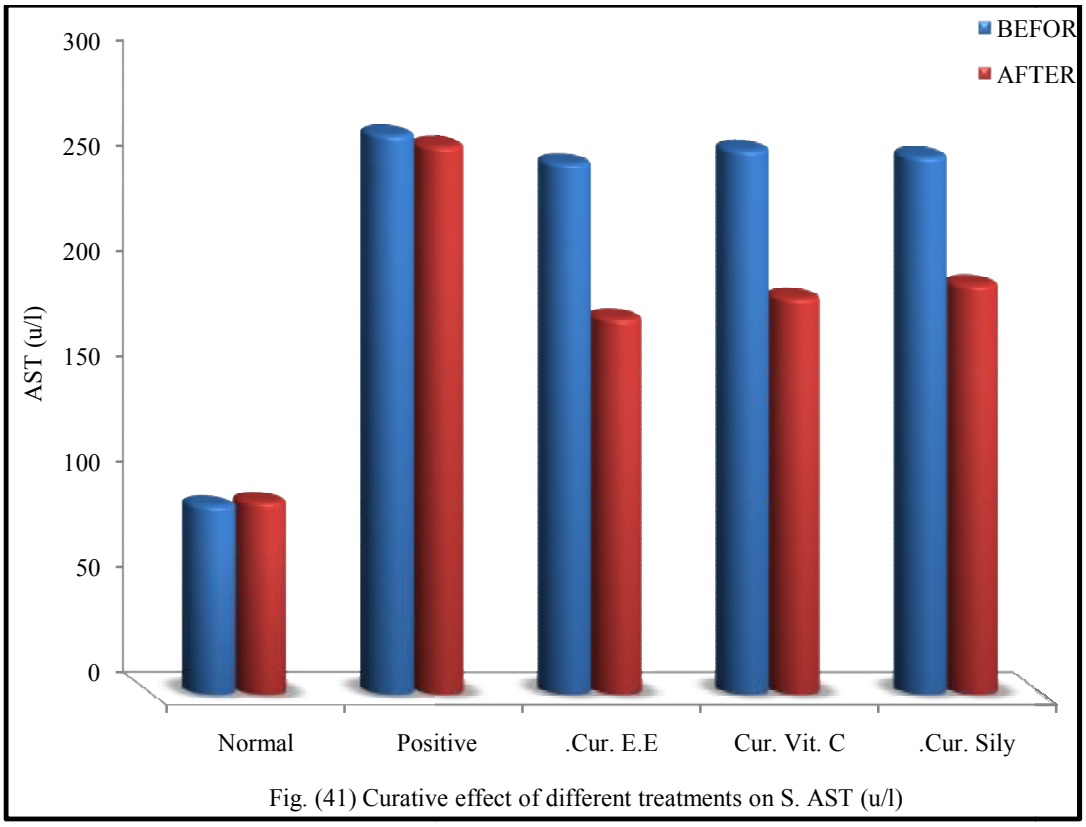


Fig. (41) Curative effect of different treatments on S. AST (u/l)

Table (24): Curative effect of different treatments on S. LDH (U/L ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After treatment	% change
Control	40.12± 6.7	38.97±8.0 †	2.86 ↓
Positive control	127.34± 10.1	130.45±12.6 a, ***	2.44 ↑
Ecballium elaterium fruit juice "100 µl"	127.52±11.7	68.53±7.3 b, ***	46.25 ↓
Vitamin C (300 mg/kg. b.w.)	124.96±9.76	85.54±9.6 b, ***	31.54 ↓
Silymarin (50 mg/kg.b.w.)	128.75±12.3	75.82±10.4 b, ***	41.11 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

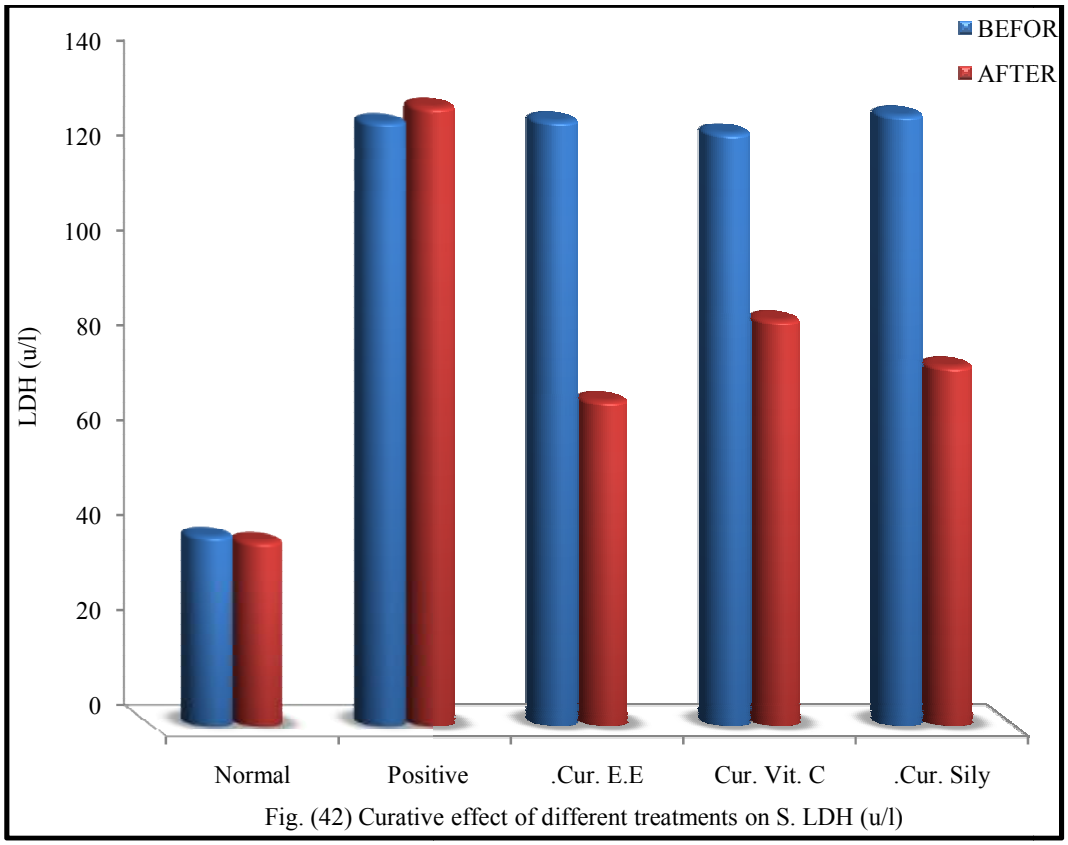


Fig. (42) Curative effect of different treatments on S. LDH (u/l)

Table (25): Curative effect of different treatments on S. G-GT (U/L ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After treatment	% change
Control	3.77±0.26	3.67±0.11 †	2.65 ↓
Positive control	13.44±4.22	12.3±2.11 a, ***	8.48 ↓
Ecballium elaterium fruit juice "100 µl"	12.69±4.87	7.24±2.15 b, **	42.94 ↓
Vitamin C (300 mg/kg.b.w.)	13.10±5.09	8.04±2.07 b, **	38.62 ↓
Silymarin (50mg/kg.b.w.)	12.72±5.23	7.92±1.13 b, **	37.73 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

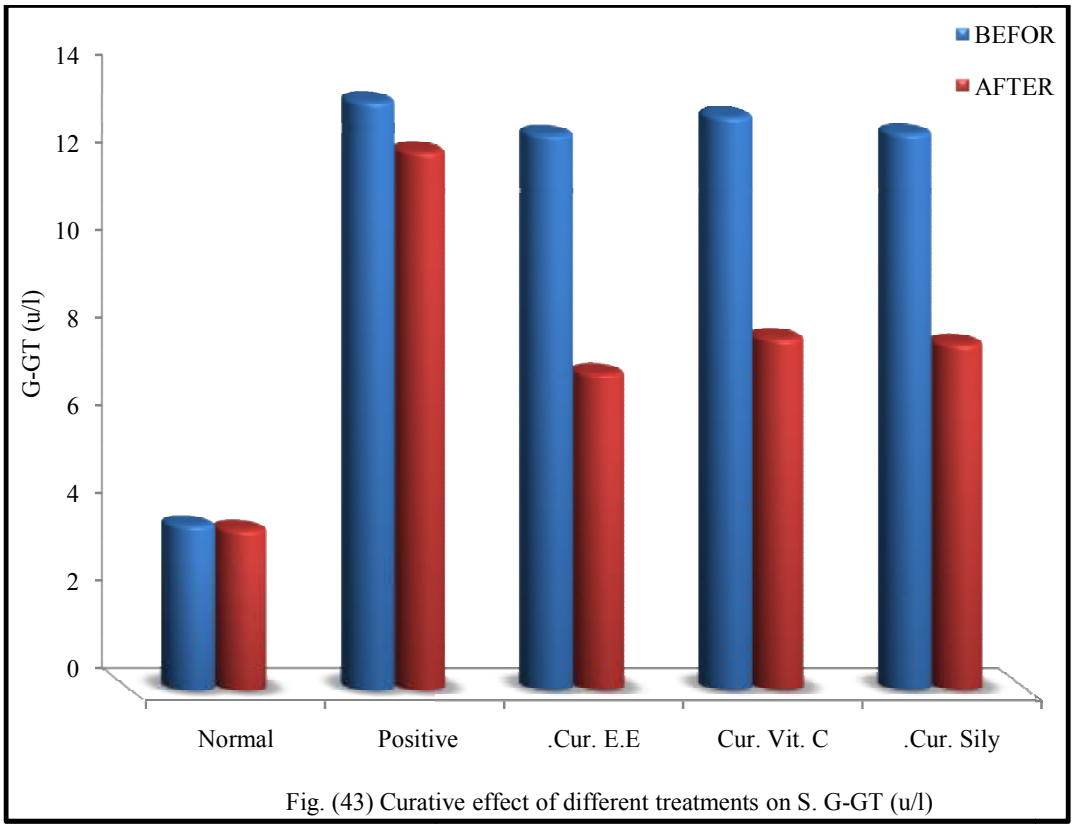


Fig. (43) Curative effect of different treatments on S. G-GT (u/l)

Table (26): Curative effect of different treatments on S. T. Protein (g/dl ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After treatment	% change
Control	9.50 ± 1.02	9.82±0.56 †	3.36 ↑
Positive control	4.07± 0.46	4.12±1.33 a, ***	1.22 ↑
Ecballium elaterium fruit juice "100µl"	4.18±1.11	7.11±0.99 b, **	70.09 ↑
Vitamin C (300 mg/kg.b.w.)	4.21±0.58	6.15±1.10 b, **	46.08 ↑
Silymarin (50 mg/kg.b.w.)	4.17±1.19	6.75±1.02 b, **	61.87 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

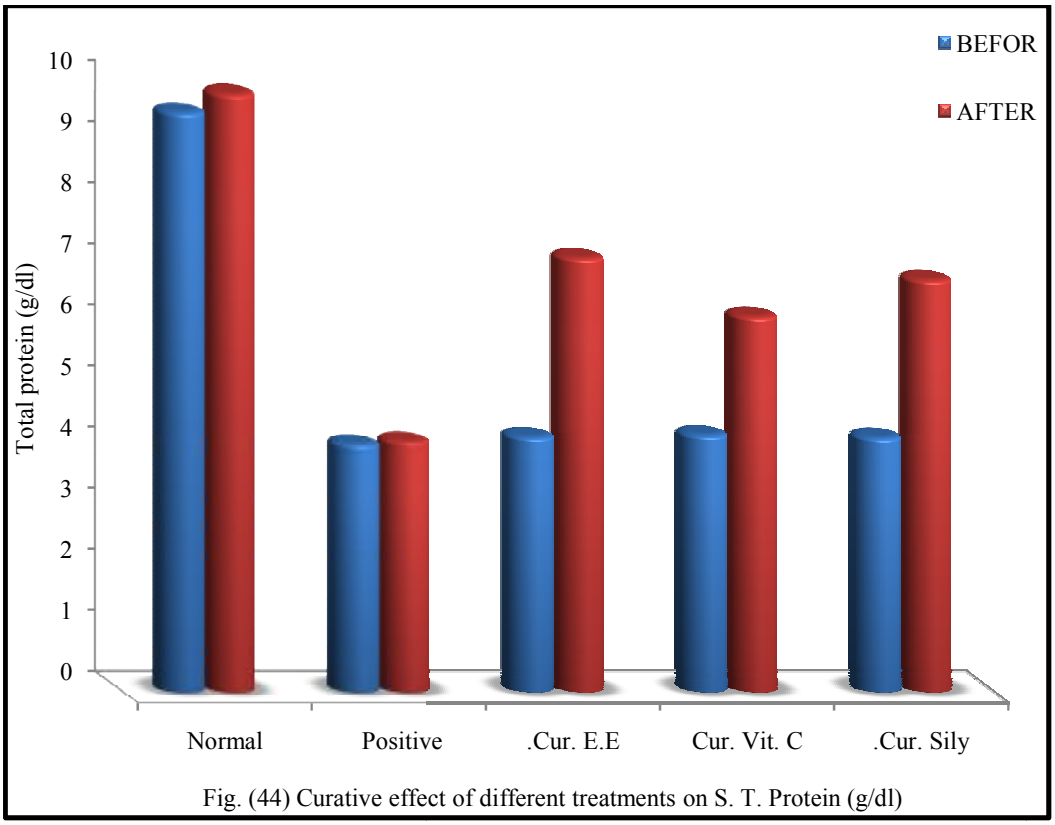


Fig. (44) Curative effect of different treatments on S. T. Protein (g/dl)

Table (27): Curative effect of different treatments on S. albumin (g/dl ±S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After treatment	% change
Control	7.55± 0.29	7.51±0.31 †	0.52 ↓
Positive control	3.11 ± 0.14	3.21±0.51 a, ***	3.21 ↑
Ecballium elaterium fruit juice "100µl"	3.31±0.23	5.60±0.29 b, **	69.18 ↑
Vitamin C (300 mg/kg.b.w.)	3.17±0.11	4.97±0.46 b, **	56.78 ↑
Silymarin (50 mg/kg.b.w.)	3.29±0.35	5.28±0.17 b, **	60.48 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

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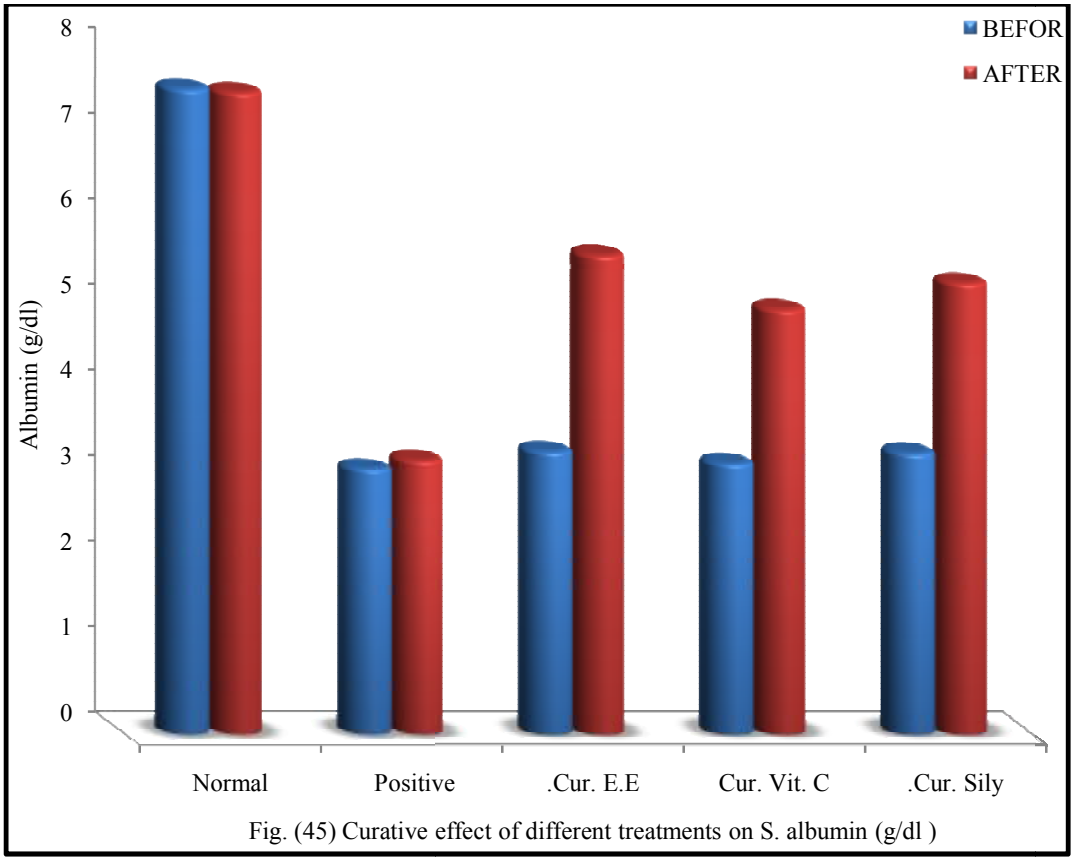

Fig. (45) Curative effect of different treatments on S. albumin (g/dl)

Table (28): Curative effect of different treatments on total bilirubin (mg/dl ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After treatment	% change
Control	0.44±0.03	0.40±0.01 †	9.09 ↓
Positive control	2.19± 0.12	2.12±0.77 a, ***	3.19 ↓
Ecballium elaterium fruit juice "100 µl"	2.09±0.34	0.83±0.11 b, ***	60.28 ↓
Vitamin C (300 mg/kg.b.w.)	1.98±0.11	1.00±0.13 b, ***	49.49 ↓
Silymarin (50 mg/kg.b.w.)	2.30±0.15	1.25±0.21 b, ***	45.65 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

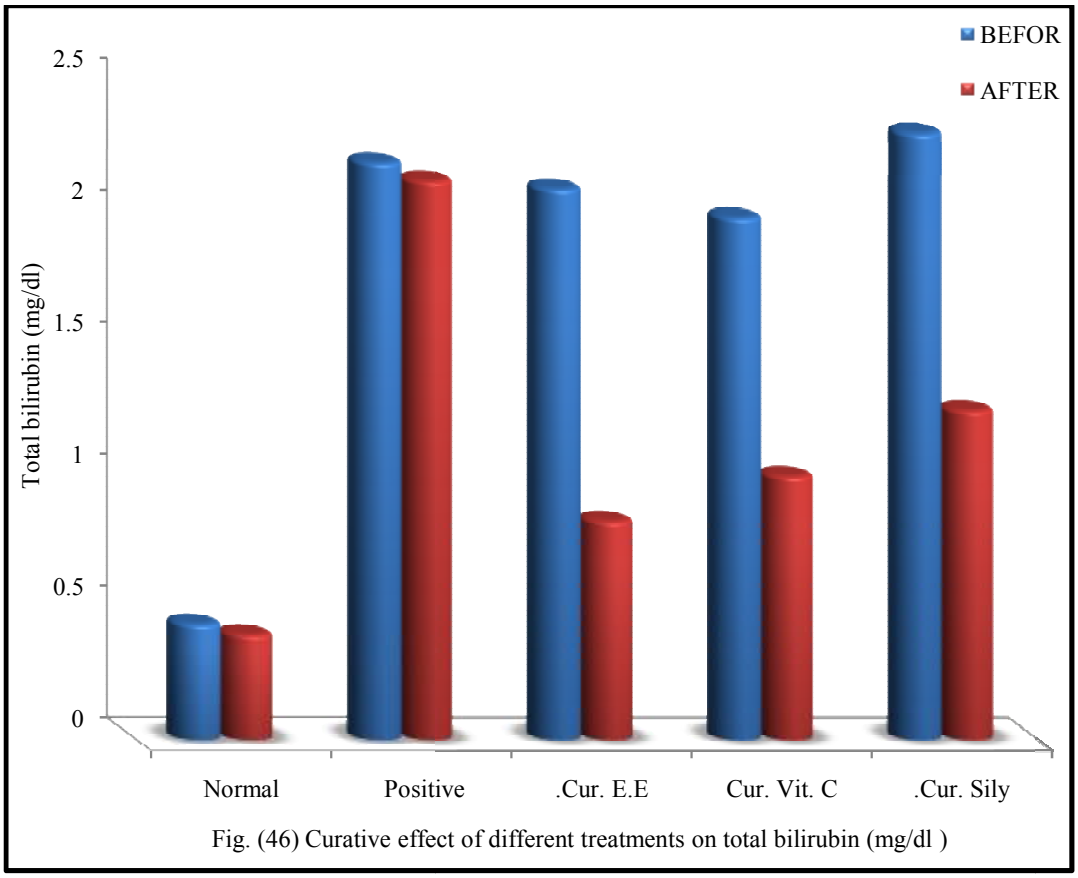

Fig. (46) Curative effect of different treatments on total bilirubin (mg/dl)

Table (29): Curative effect of different treatments on S. ALP (U/L ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After treatment	% change
Control	146.4± 7.93	144.47±8.95 †	1.31 ↓
Positive control	266.6± 14.1	263.68±13.11 [a, ***]	1.09 ↓
Ecballium elaterium fruit juice "100µl"	272.8±8.34	190.94±6.42 [b, ***]	30 ↓
Vitamin C (300 mg/kg.b.w.)	267.3±10.1	215.32±8.10 [b, ***]	19.44 ↓
Silymarin (50 mg/kg.b.w.)	275.9±6.22	200.97± 9.02 [b, ***]	27.116 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with acetaminophen positive group.

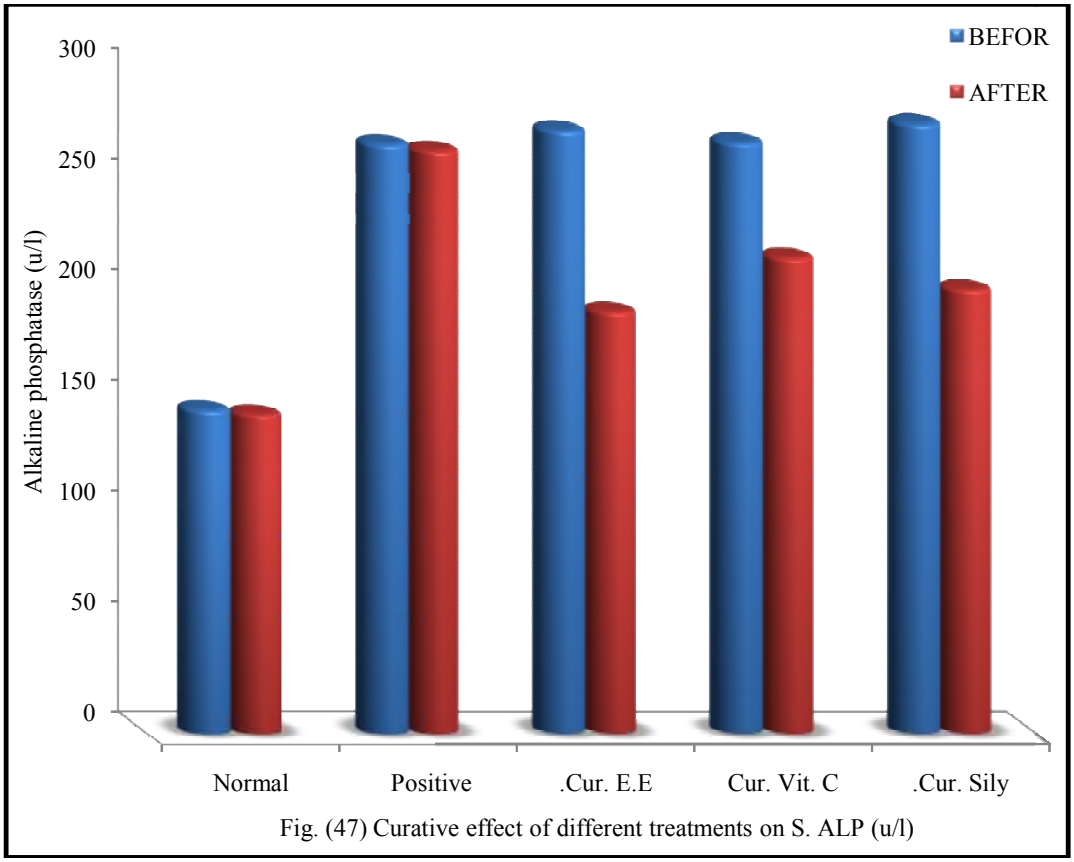

Fig. (47) Curative effect of different treatments on S. ALP (u/l)

Table (30): Curative effect of different treatments on S. creatinine (mg/dL ± S. D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After treatment	% change
Control	0.28 ± 0.01	0.29±0.09 †	3.57 ↑
Positive control	1.29±0.04	1.23 ±0.21 a, ***	4.65 ↑
Ecballium elaterium fruit juice "100µl"	1.17±0.33	0.63±0.11 b, ***	46.15 ↓
Vitamin C (300 mg/kg.b.w.)	1.20±0.75	0.87±0.05 b, ***	27.5 ↓
Silymarin (50 mg/kg.b.w.)	1.24±0.82	0.65±0.06 b, ***	47.58 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

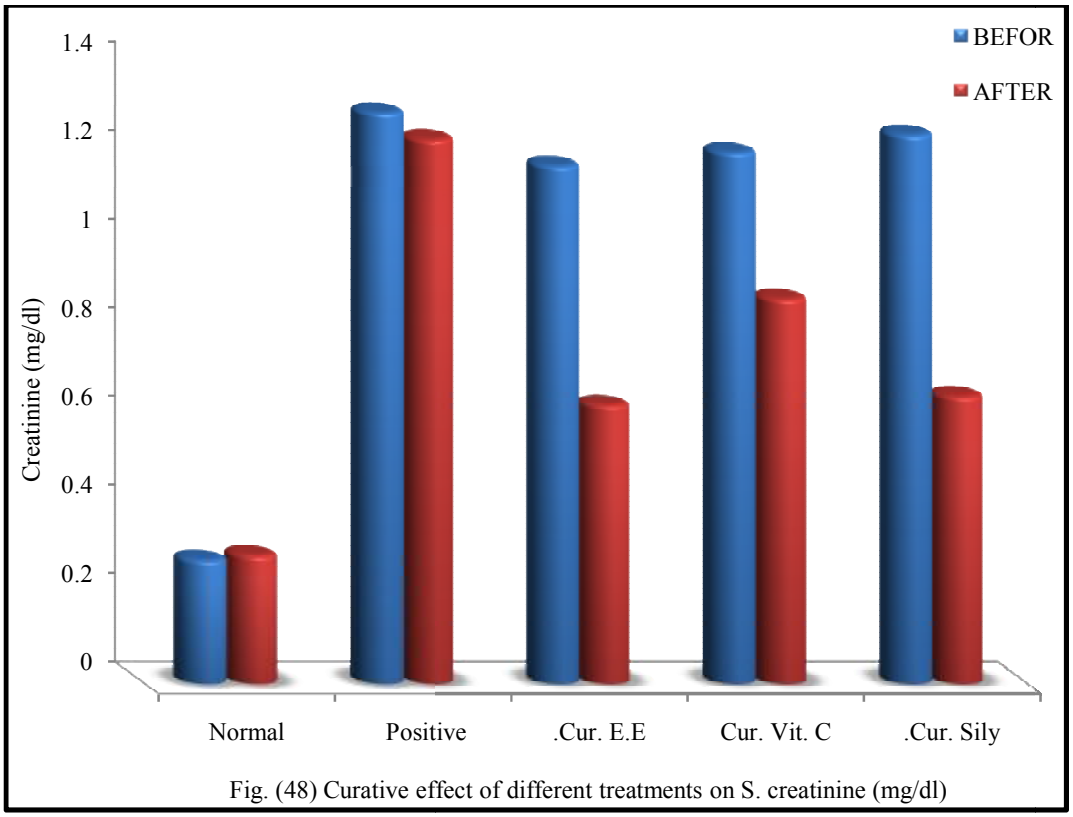

Fig. (48) Curative effect of different treatments on S. creatinine (mg/dl)

Table (31): Curative effect of different treatments on blood urea (mg/dL ± S. D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After treatment	% change
Control	20.6±3.75	20.81±2.11 †	1.01 ↑
Positive control	42.77±7.33	45.12±11.3 a, ***	5.49 ↑
Ecballium elaterium fruit juice "100µl"	47.85± 7.01	35.55±4.44 b, *	25.70 ↓
Vitamin C (300 µg/kg.b.w.)	43.10± 5.62	31.27±5.39 b, *	27.44 ↓
Silymarin (50 mg/kg.b.w.)	41.36± 7.44	37.76±4.09 b, †	8.70 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

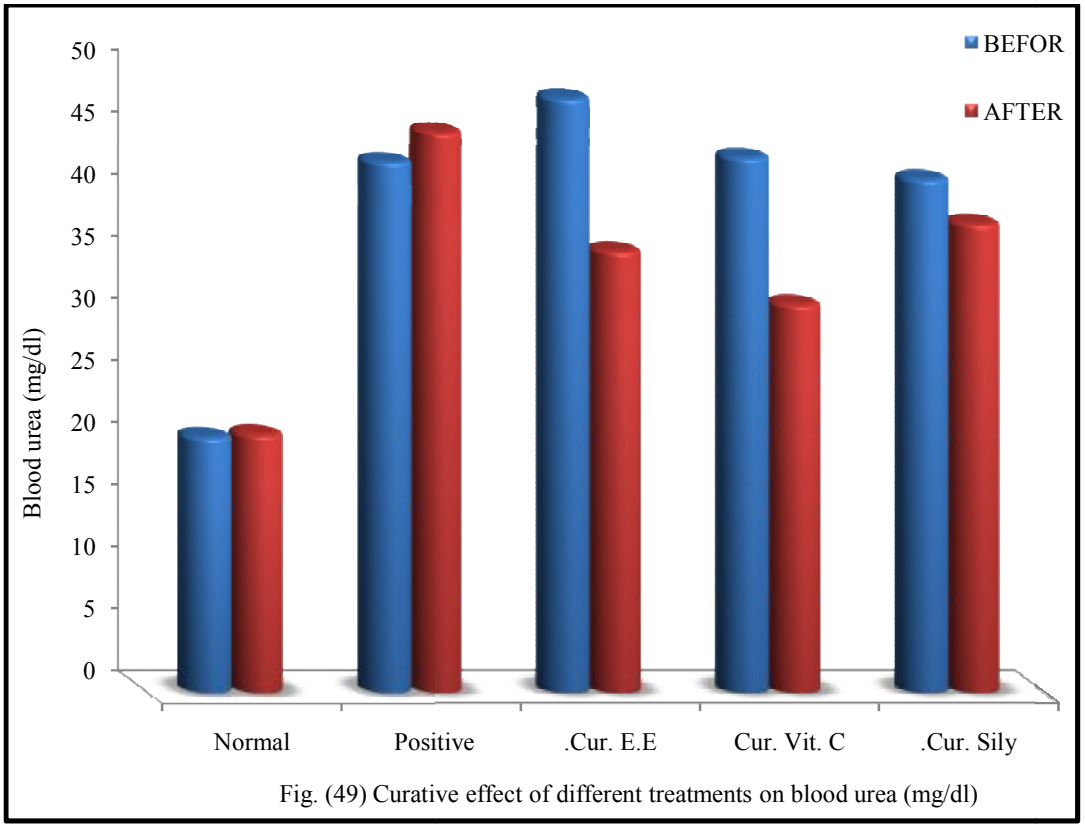

Fig. (49) Curative effect of different treatments on blood urea (mg/dl)

Table (32): Curative effect of different treatments on plasma GR (u/l ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After treatment	% change
Control	28.58±2.99	29.75±3.05 †	4.09 ↑
Positive control	8.97±2.41	10.77±1.57 a, **	20.06 ↑
Ecballium elaterium fruit juice "100 µl"	7.73±3.69	20.91±3.53 b, *	161.19 ↑
Vitamin C (300 mg/kg.b.w.)	9.31±1.95	16.61±2.87 b, †	78.41 ↑
Silymarin "50 mg/kg b.w."	9.88±2.71	18.37±1.77 b, †	85.93 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

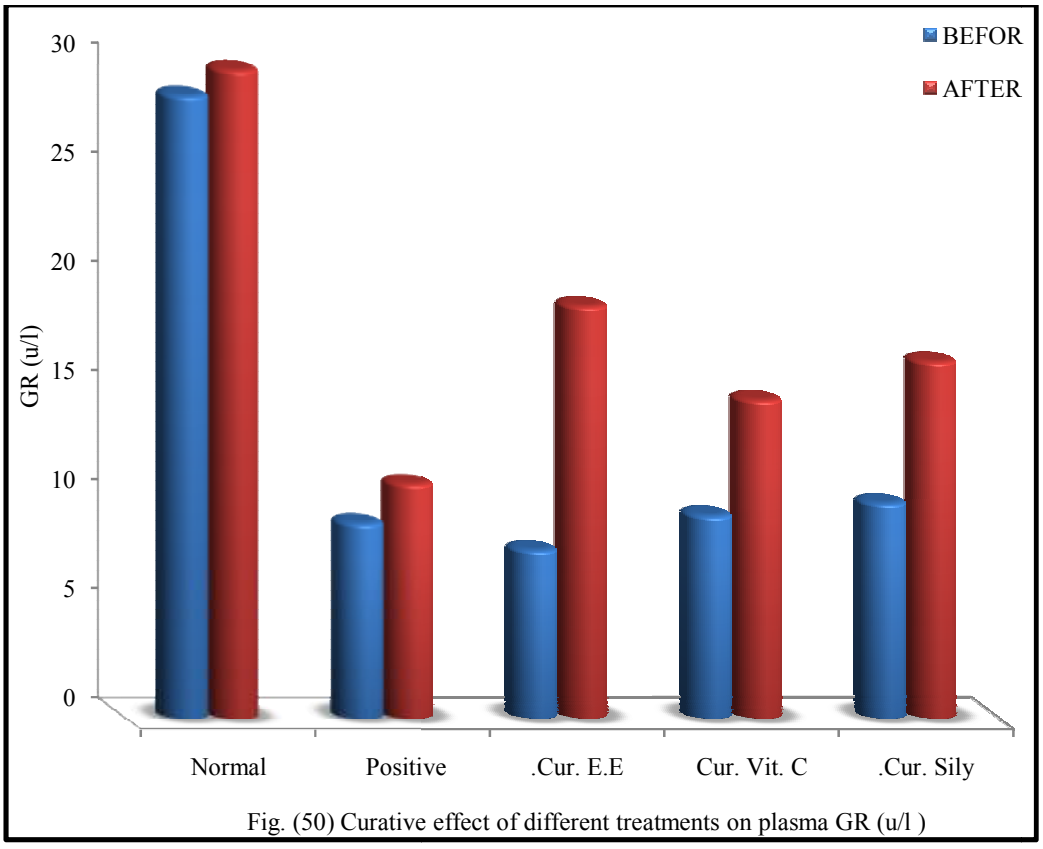

Fig. (50) Curative effect of different treatments on plasma GR (u/l)

Table (33): Curative effect of different treatments on plasma GP_X (mu/ml ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After treatment	% change
Control	32.14±2.65	35.17±2.79	9.42↑
Positive control	10.74±2.93	11.63 ± 2.67 [a, **]	8.28↑
Ecballium elaterium fruit juice"100 µl"	13.12±1.49	20.17±2.26 [b, *]	53.73↑
Vitamin C (300 mg/kg.b.w.)	12.66±2.74	16.87±3.74 [b, †]	33.25↑
Silymarin "50 mg/kg b.w."	11.56±2.73	19.71±1.33 [b, †]	70.50↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with acetaminophen positive group.

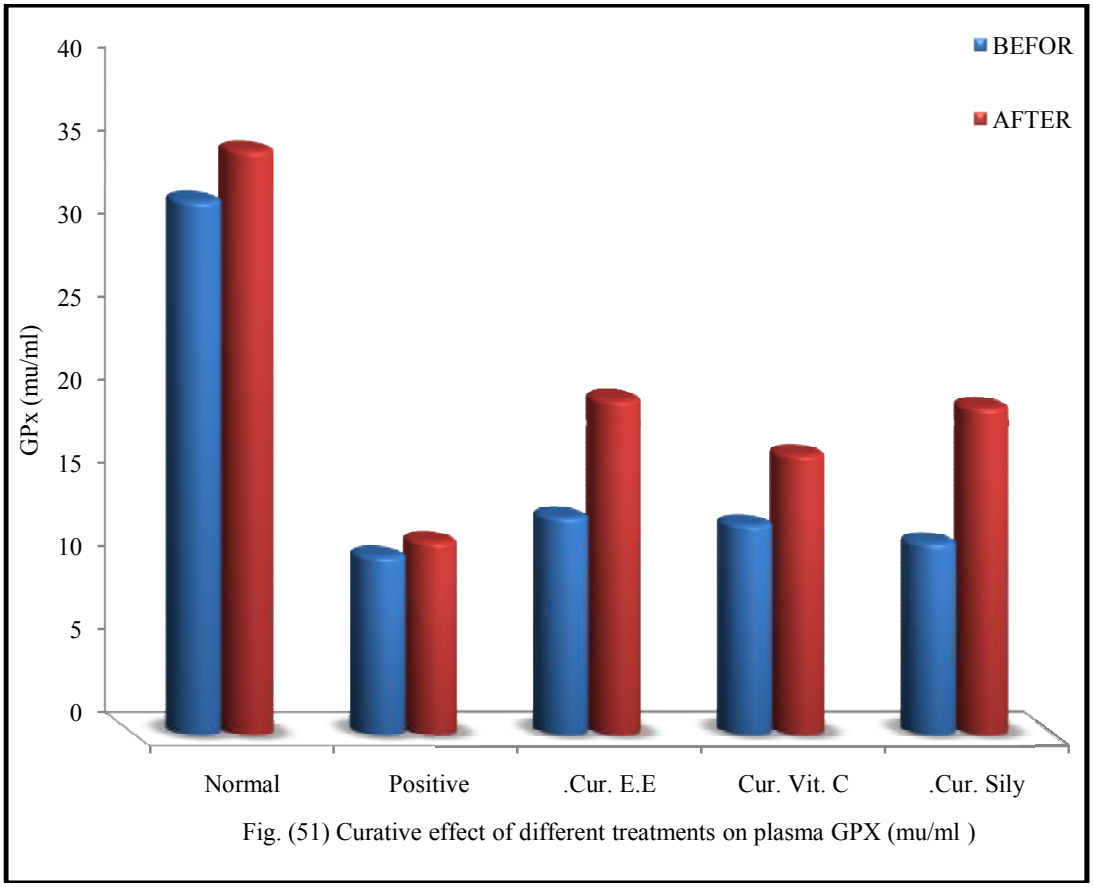

Fig. (51) Curative effect of different treatments on plasma GPX (mu/ml)

Table (34): Curative effect of different treatments on plasma CAT (u/l ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After treatment	% change
Control	49.67±4.37	52.62±5.49 †	5.93 ↑
Positive control	20.93±2.05	19.87±3.17 a, ***	5.06 ↓
Ecballium elaterium fruit juice "100 µl"	20.76±2.47	34.87±3.71 b, *	67.96 ↑
Vitamin C (300 mg/kg.b.w.)	22.56±2.47	28.75±1.97 b, †	27.43 ↑
Silymarin (50 mg/kg.b.w.)	20.89±2.13	32.63±2.16 b, *	56.19 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with acetaminophen positive group.

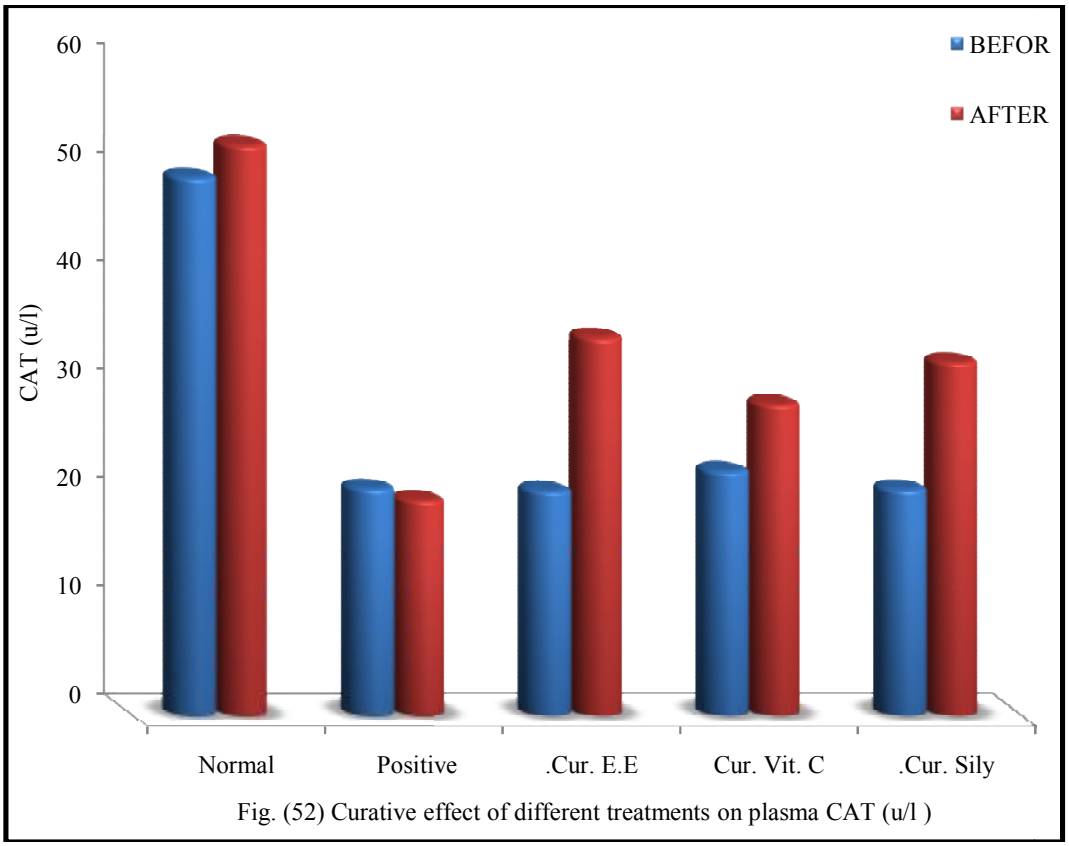

Fig. (52) Curative effect of different treatments on plasma CAT (u/l)

Table (35): Curative effect of different treatments on plasma SOD (u/mol ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After treatment	% change
Control	15.33±3.97	16.75±2.17 †	9.26 ↑
Positive control	4.95± 2.16	4.13±1.57 [a, ***]	16.56 ↓
Ecballium elaterium fruit juice "100 µl"	4.79±3.01	7.86±1.21 [b, ***]	64.09 ↑
Vitamin C (300 mg/kg.b.w.)	5.17±2.73	5.81±1.25 [b, **]	12.37 ↑
Silymarin (50 mg/kg.b.w.)	4.53±3.79	6.22±3.31 [b, **]	37.30 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with acetaminophen positive group.

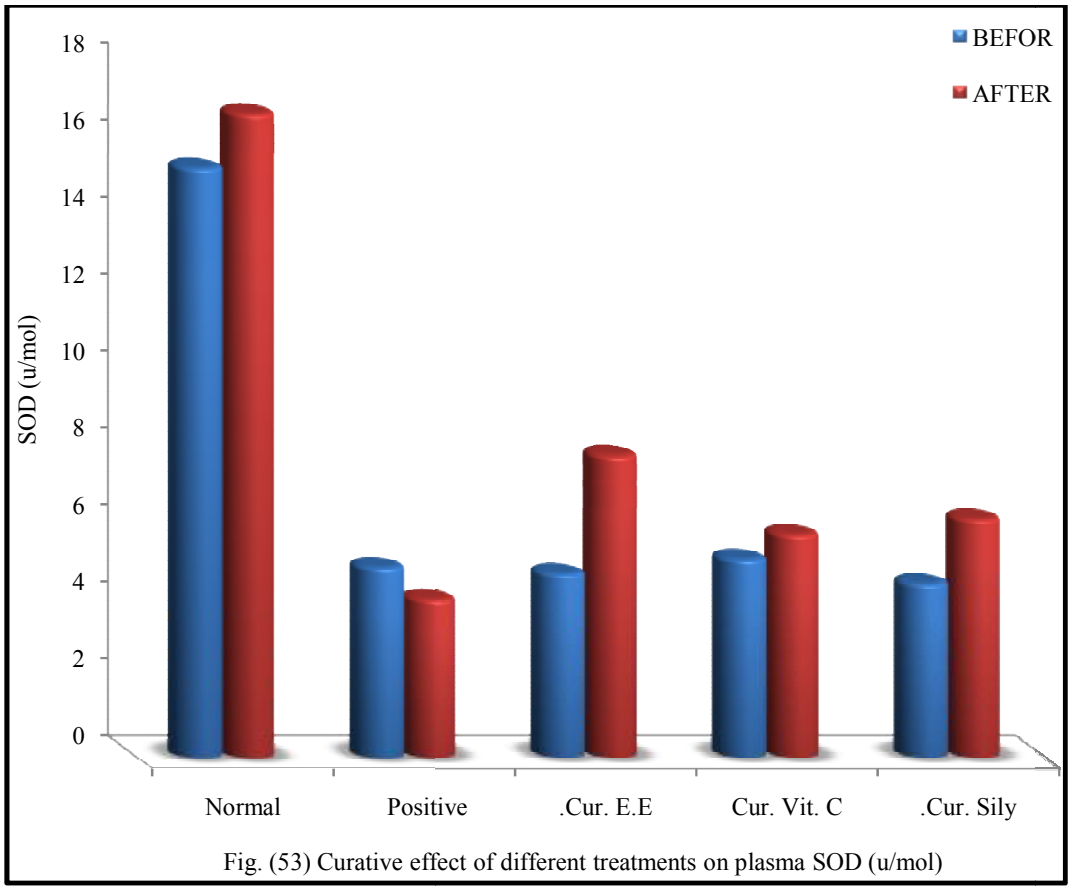

Fig. (53) Curative effect of different treatments on plasma SOD (u/mol)

Table (36): Curative effect of different treatments on plasma MDA (nmol/ml ± S.D) and % change from the corresponding control during the induction of hepatotoxicity for 3 weeks in male albino rats.

Animal groups	Before treatment (After induction)	After treatment	% change
Control	20.17±3.55	21.07±2.64 †	4.46 ↑
Positive control	48.20±2.56	50.15±2.52 a, ***	4.04 ↑
Ecballium elaterium fruit juice "100 µl"	49.37±3.27	35.41±2.59 a, *	28.27 ↓
Vitamin C (300 mg/kg.b.w)	46.81±3.13	33.71±3.05 a, *	27.98 ↓
Silymarin (50 mg/kg.b.w.)	51.79±2.11	29.31±1.13 a, **	43.40↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group ; b compared with acetaminophen positive group.

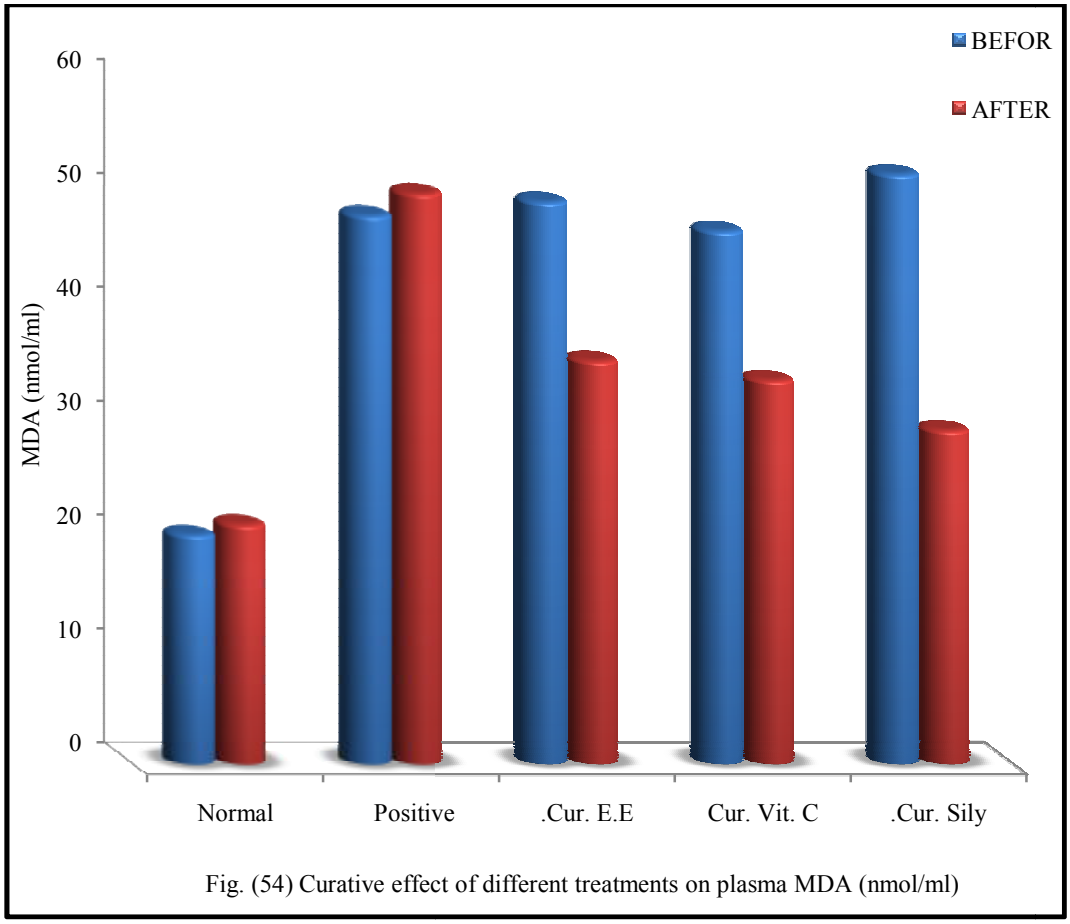

Fig. (54) Curative effect of different treatments on plasma MDA (nmol/ml)

9. Histopathology of the liver:

The results of light microscopy examination of the transverse section of control, acetaminophen -treated and treated rats liver are shown in Figs. (55, 56, 57, 58, 59)

Figure (55) shows the liver cells of rat in the normal group, from that image it can be observed that the normal liver architecture of hepatocytes, the portal vein has a regular shape; overall, a healthy set of cells can be observed.

In the rats treated with acetaminophen alone, induced marked histopathological lesion which was characterized by diffuse ballooning degeneration, dusty and cloudy swelling, fatty degeneration, hepatocellular necrosis, and irregular appearance due to cell damage were seen in fig. (56) when compared to the hepatocytes architecture of normal liver.

The hepatocytes distortion was ameliorated where nucleases are at recovery stages and absence of joined nuclease. Sinusoids are slowly recovering hepatocytes were being transformed to normal shape appearance in *Ecballium elaterium* "fruit juice" at 100 µl in post treated liver. The histopathological examination thus verified the hepatoprotective effect of *Ecballium elaterium* "fruit juice" against the acetaminophen induced hepatotoxicity. Also vitamin C and silymarin exhibit hepatoprotective effect at the 300 mg/kg and 50 mg/kg respectively as illustrated in figs. (58, 59).

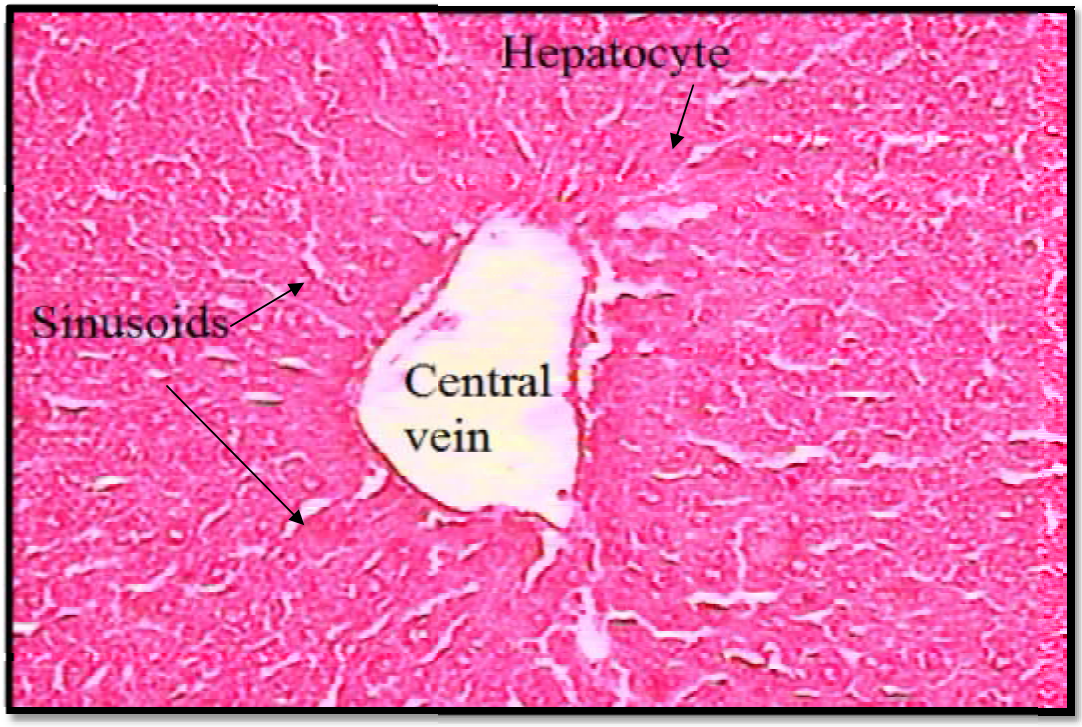

Fig. (55): Light microscopy of liver cell of normal rat.

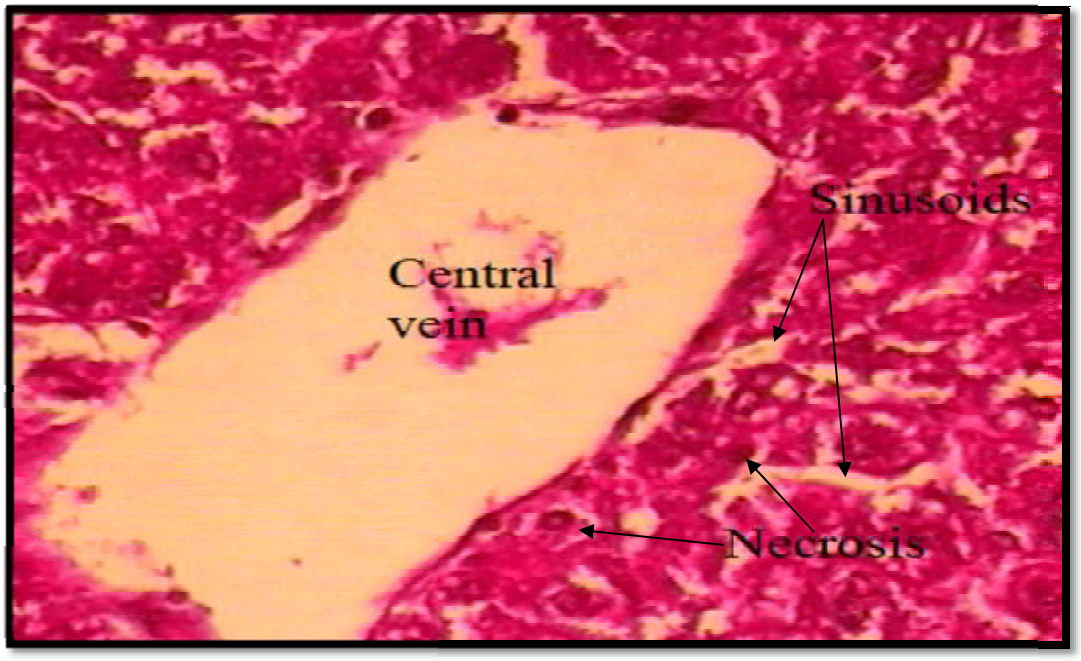

Fig. (56): Light microscopy of liver cell of positive rat treated by 400mg/kg of acetaminophen.

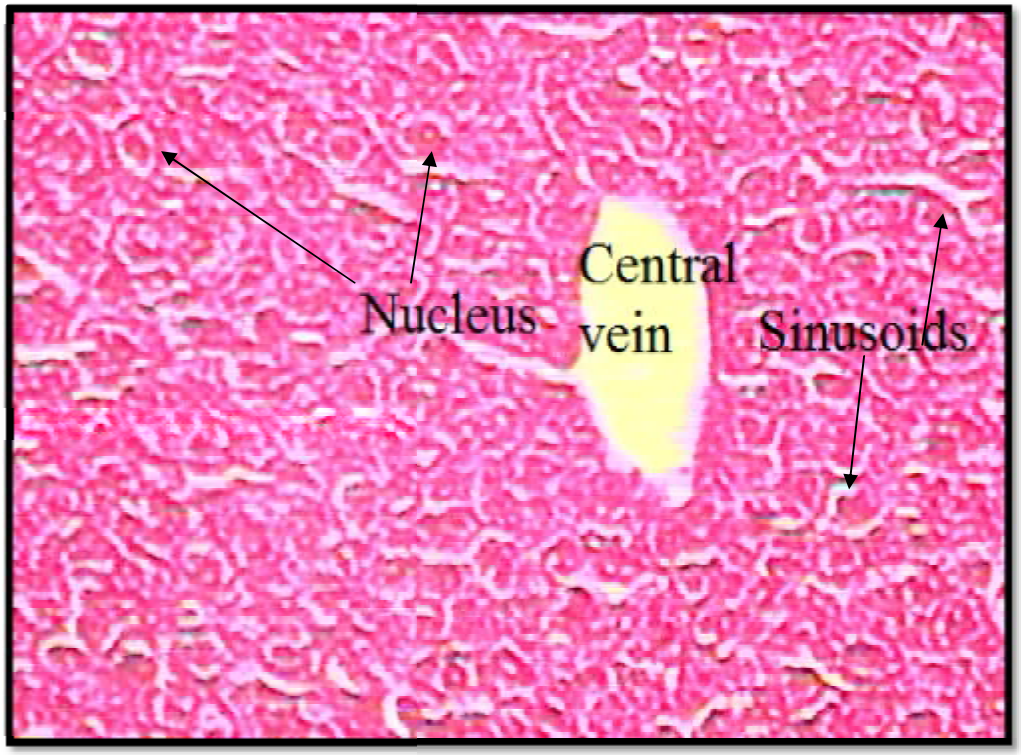

Fig. (57): Light microphotographs of liver cell treated by *Ecballium elaterium* "fruit juice" at 100 µl.

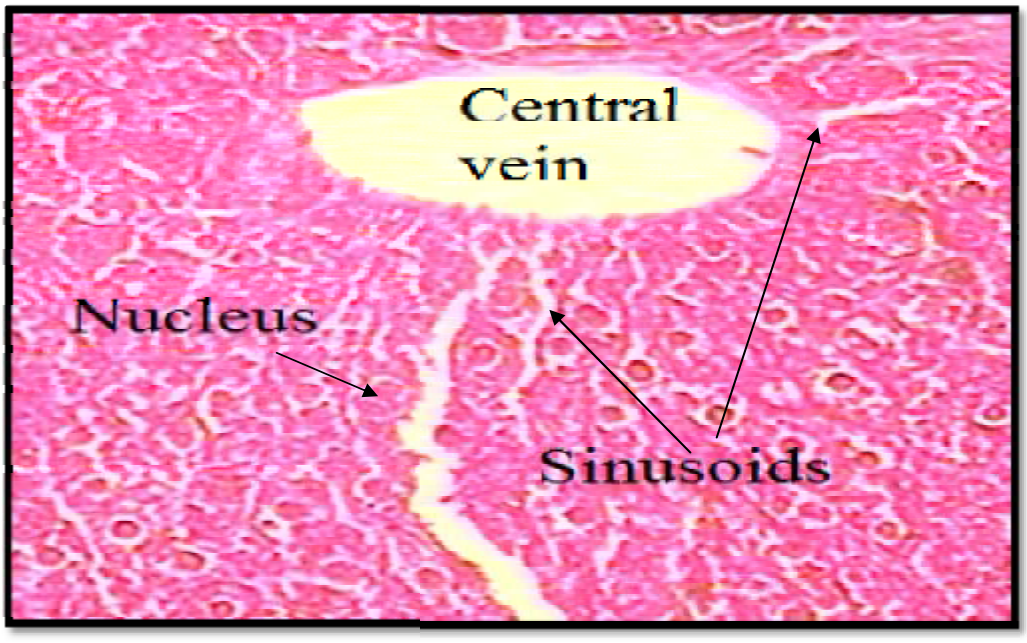

Fig. (58): Light microphotographs of liver cell treated by vitamin C at 300 mg/kg.

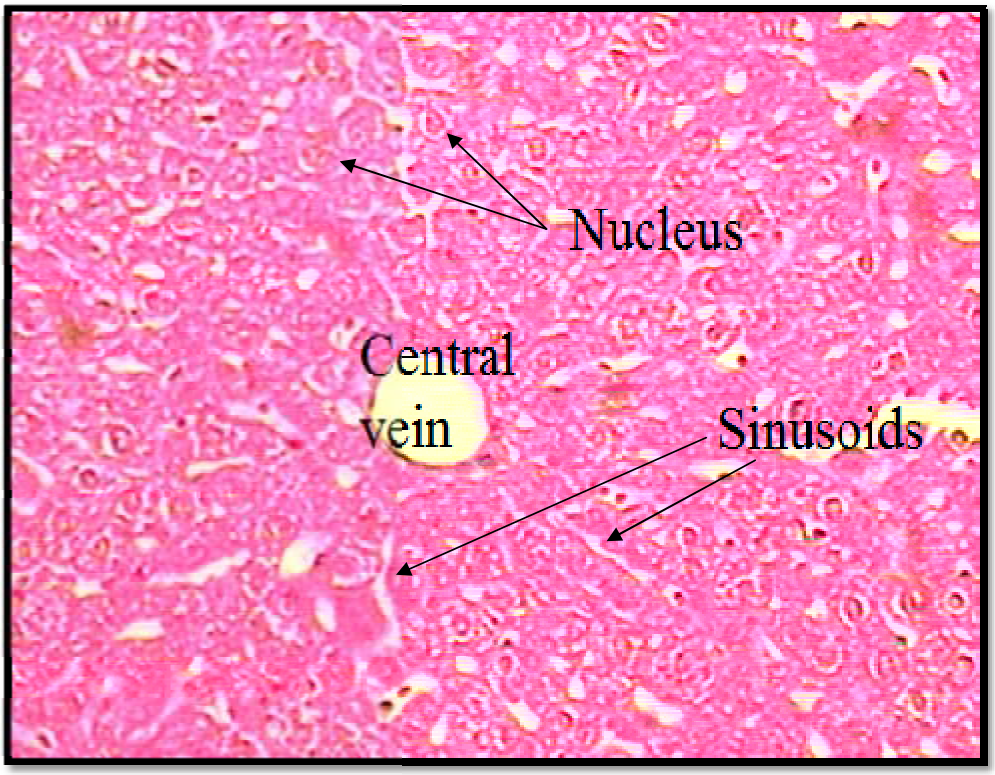

Fig. (59): Light microphotographs of liver cell treated by silymarin at 50 mg/kg.

DISCUSSION

1. Induction of hepatotoxicity:

The liver is the vital organ of paramount importance involved in the maintenance of metabolic function and detoxification from the exogenous and endogenous challenges, like xenobiotic, drugs, viral infection and chronic alcoholism.

If during all such exposures to the above mentioned challenges the natural protective mechanisms of the liver are overpowered, the result is hepatic injury. Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like serum (ALT, AST, ALP) and bilirubin are elevated **[145]**.

Acetaminophen PCM-induced acute hepatocellular injury as an experimental model of drug-induced acute hepatic necrosis is well-established. The mechanism by which PCM overdose leads to hepatocellular injury and death involves its conversion to a toxic highly reactive and cytotoxic intermediate metabolite, N-acetyl-para-benzoquinonimine (NAPQI), **[146]**. During metabolism which is formed by two-electrons oxidation **[147]**.

Normally, PCM is primarily metabolized via cytochrome P_{450} 2E1 to form the highly electrophilic NAPQI which is eliminated by conjugation with glutathione (GSH). However, during PCM poisoning, hepatocellular levels of GSH are markedly depleted. GSH depletion makes the hepatocytes susceptible to the toxic effects of NAPQI. Its depletion allows NAPQI to binds irreversibly and covalently to cell macromolecules resulting in intrahepatic enzymatic system dysfunction **[146]**.

Primary cellular targets have been postulated to be mitochondrial proteins, with resulting loss of energy production, as well as other factors recently identified that contribute to the toxicity. These factors include oxidative stress, nitrotyrosine formation, inflammatory cytokines, and the possible importance of mitochondrial permeability transition **[147]**. This process disrupts homeostasis and initiates apoptosis " programmed cell death" leading to tissue necrosis and ultimately to organ dysfunction **[148]**.

Several laboratories have studied the role of macrophage activation in acetaminophen toxicity. Kupffer cells are the phagocytic macrophages of the liver. When activated, Kupffer cells release numerous signaling molecules, including hydrolytic enzymes, nitric oxide, and superoxide. Kupffer cells may also release a number of inflammatory cytokines including TNF-α **[149]**.

The rats were acclimatized to laboratory condition for 10 days before commencement of experimental. Acetaminophen was orally administered to animals with dose 400 mg/kg at every 48 h for 22 days.

In the assessment of liver damage by using acetaminophen (PCM) induced liver injury in the rat as a model, was measured by using biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT) *aka* (AST), serum glutamate pyruvate transaminase (SGPT) *aka* (ALT) alkaline phosphatase (ALP), bilirubin (BRN), albumin, and Gama-Glutamyl transferase (G-GT) **[150, 151, 152]**.

Because liver damage arising from necrosis or membrane damage normally releases the enzymes into circulation; therefore, measurement of these enzymes in serum gives an indication of the health status of the liver. High levels of AST indicate liver damage, as that due to viral hepatitis. ALT catalyses the conversion of alanine to pyruvate and α- ketoglutarate to glutamate, and is released in a similar manner and thus ALT is more specific to the liver, and consider as a better parameter for detecting liver injury. It is known that an increase in the enzymatic activity of ALT and AST in the serum directly reflects a major permeability or cell rupture. An increase in AST and ALT, a hepatospecific enzyme that is principally found in the cytoplasm in the rats following administration of a hepatotoxin is attributed to the increased release of enzymes from the damaged liver parenchymal cells **[153, 154]**.

Serum ALP, bilirubin and albumin levels on other hand are related to the function of hepatic cells **[155]**.

Administration of PCM caused a significant elevation of ALP, and total bilirubin. Serum alkaline phosphatase increases to some extent in most types of liver injury. Bile acids induce alkaline phosphatase synthesis and exert a deterrent effect. The highest concentrations are observed with cholestatic injuries also serum albumin, the major plasma protein synthesized in the human liver, is a clinically useful marker of hepatic synthetic function. in the liver constitutes a major part of the total proteins in the body and the other part being globulin. A low serum albumin concentration suggests chronic liver disease **[156]**. Bilirubin concentration has been used to evaluate chemically induced hepatic injury.

Besides its various normal functions, the liver excretes the breakdown product of hemoglobin, namely bilirubin, into bile. It is well known that necrotizing agents like acetaminophen produce sufficient injury to the hepatic parenchyma to cause large increases in bilirubin content. The measurement of serum γ-glutamyl transferase is a frequently used parameter of liver diseases. The lactate dehydrogenase (LDH) is also elevated in diseases of the liver when disease or injury affects tissues containing LDH, the cells release it into the bloodstream **[157]**.

Serum LDH, a cytoplasmic marker enzyme and G-GT, are the well known indicators of cell and tissue damage by toxic substances and their levels are also substantially increased in acetaminophen-intoxicated rats. LDH is an intracellular enzyme, the increase of which in serum is an indicator of cell damage **[158]**.

In the present study increased levels of serum hepatic markers suggested that an extensive liver injury was occasioned by acetaminophen due to increased lipid peroxidation which have the ability to cause membrane damage. Acetaminophen causes structural and functional damage to the cell membrane and increased the membrane permeability leading to the leakage of the hepatic enzymes into the blood. It is well established that acetaminophen toxicity significantly elevated the serum hepatic marker enzymes **[159, 160]**.

2. Effect of *Ecballium elaterium* "fruit juice" against hepatotoxicity.

The effects of *E. elaterium* "fruit juice on acetaminophen induced hepatotoxicity showed that decrease in the level of AST, ALT, ALP, LDH, G-GT, total bilirubin and elevated total protein, and albumin. While the acetaminophen-treated group showed elevated levels of these enzymes, confirming that acetaminophen caused liver injury at high doses. except total protein and albumin, decline in total protein content and albumin can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. decrease in the level of total proteins and albumin was observed which may be due to acute hepatocellular damage and biliary obstruction, the biochemical parameters of the *E. elaterium* "fruit juice" treated group were higher than those of the control group, but it showed much lower than of the acetaminophen -treated group.

The decrease in the serum levels of these enzymes might be due to the presence of various phenolic, flavonoid compounds and triterpenoids "Cucurbitacin" in the fruit juice that may be responsible for the protective effect on acetaminophen induced liver damage in rats. The most active constituents of *Ecballium elaterium* "fruit juice". Cucurbitacin seems to be responsible for the major pharmacological and biological effects of *E. elaterium* plant. For example, due to its strong bitter taste, cucurbitacin acts as purgative agent by stimulating gastric secretion. Also it has been found to decrease the damage in the chronic hepatitis and is responsible for the antimicrobial, antifungal and anti-inflammatory activity of cucurbitacin B isolated from the juice of *E. elaterium* **[161]**.

Attainment of normal levels of total protein and albumin in *E. elaterium* treated rats confirms the hepatoprotective effect of the *E. elaterium*, that may result in better protection against liver disorders, our results are in agreement with this finding which proved by **(Agil A, et al.,)**. Who reported that the antihepatotoxic activity of (dried juice of the fruits of *E. elaterium*) was studied against CCl_4-induced hepatotoxicity. Pre-treatment and post-treatment with *E. elaterium* reduced CCl_4-hepatotoxicity, as shown reduction in the increase of serum of GPT level. *E. elaterium* "fruit juice" has preventive and curative effects against CCl_4-induced hepatotoxicity **[162]**.

Cucurbitacin B isolated from the juice of *E. elaterium* has been shown to be effective chronic hepatitis by normalizing hepatic protein levels, stimulating cellular immunity functions, decrease levels of GPT in rats with experimental fatty liver (CCl_4) thus decrease hepatic damage. In chronic experiments it has been shown to prevent hepatitis and cirrhosis **[163]**.

As mentioned above and according to our phytochemical screening the phenolic and flavonoid content of fruit juice support its biological activity and its utilizing in preventative and treatment of cardiovascular disease, cancer, inflammatory conditions and liver disease. The flavonoids substances were considered to be responsible for the free radical scavenging properties of *E. elaterium* "fruit juice".

Vitamin C used as reference in scavenging effect tests also reported to have strong hepatoprotective effect **[164]**.

In the present study treatment with vitamin C (300 mg/kg) reduced the serum levels of liver enzymes toward the respective normal value this clearly indicates that the vitamin C has stabilizes the plasma membrane as well as helped in healing of the hepatic tissue damage, resulting in lower levels of , ALT, AST, ALP, LDH, G-GT, than the acetaminophen-treated group, hepatotoxic rats in both prophylactic and curative groups especially when compared with the positive group acetaminophen-treated group. Serum ALP, and total bilirubin levels are also related to the status and function of hepatic cells. The present study indicates that vitamin C at this dose reduces the serum ALP, and total bilirubin.

The vitamin C (300 mg/kg) dose improve the secretory mechanism of hepatic cells. Protective effect of vitamin C which is clearly evident from the restoration of decrease in the level of total proteins and albumin by increase the level of total protein and albumin similar to that of *E. elaterium* "fruit juice" treated group. The possible mechanism by which vitamin C exhibited significant protection against acetaminophen induced hepatotoxicity these results suggested that although vitamin C may enhance recovery from hepatotoxicity associated with exposure to acetaminophen in male albino rats. Vitamin C, as an antioxidant agent, may have inhibition the chain reactions of acetaminophen-generated free radicals or scavenged the reactive free radicals before reaching their hepatic targets in animal also we can notice these effects of vitamin C in

curative group at the same dose **[165]**. These results is in agreement with **(Adejuwon A. Adeneye et al., 2008)**.

Oral administration of vitamin C ameliorate hepatotoxic effect of repeated high dose of acetaminophen, effect which was possibly mediated via free radical scavenging and/or inhibition of free radical generation.

The hepatoprotective potency of silymarin also recorded in curative procedure where silymarin significantly restores the changes of ALT, AST, ALP, LDH, G-GT. Due to its antioxidant effect and its ability to act as a free radical scavenger, thereby protecting membrane permeability **[166]**.

Silymarin has antihepatotoxic activity against carbon tetrachloride induced hepatotoxicity in albino rats. Silymarin protects against increase in serum ALT, AST and alkaline phosphates **[167]**.

The protective action of silymarin is associated with its antioxidant properties, as it possibly acts as a free radical scavenger, an inhibitor of lipid peroxidation and a plasma membrane stabilizer **[168]**.

This is view that serum levels of transaminases return to normal with the healing of hepatic and the regeneration of hepatocytes. The lowered level of total protein and albumin recorded in the serum acetaminophen treated rats reveal the severity of hepatopathy.

Silymarin treated rats maintained near the normalcy of total protein and albumin level. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process. The results that we get them are in agreement with **[169]**.

Hepatotoxicity is clear that when acetaminophen was used to induce liver toxicity there is a substantial increase in the serum bilirubin. Any decrease in the activity of bilirubin would indicate reversed of induced liver toxicity. The results indicated that the silymarin showed significantly reduced the elevated levels of serum bilirubin when compared with acetaminophen treated group. From these results, it was suggested that silymarin protects liver cells from acetaminophen-induced liver damage by its antioxidative effect on hepatocytes, hence diminishing or eliminating the harmful effects of toxic metabolites of acetaminophen.

During the metabolism process of acetaminophen via cytochrome P-450 to form the highly electrophilic NAPQI. NAPQI can rapidly react with glutathione (GSH) and lead to a 90% total hepatic GSH depletion in cells and mitochondria which can result in hepatocellular death and mitochondrial dysfunction **[170]**.

And depletion of the antioxidant enzymes (SOD, CAT, GPx) that result in oxidative stress **[171]**.

An elevation in the levels of MDA and finally lead to oxidative stress. Glutathione (GSH) is one of the most abundant naturally occurring tripeptide, non-enzymatic biological antioxidant present in liver **[172]**.

Its functions are concerned with the removal of free radicals such as H_2O_2 and superoxide radicals, maintenance of membrane protein, detoxification of foreign chemicals and biotransformation of drugs **[173]**.

In the present study, the depressed levels of GSH in acetaminophen toxicity might increase the susceptibility of the liver to free radical damage. Glutathione Peroxidase GPx is a selenoenzyme, which plays a major role in the reduction of H_2O_2 to non-toxic products **[174]**.

In this study, the observed decrease in GPx an oxidative type of injury with acetaminophen-induced hepatotoxicity. The decrease in GPx is potentially ascribable to inactivation by the increase in reactive oxygen species (ROS) or lipid peroxides when oxidative damage is extreme **[175]**.

Glutathione reductase (GR) is the enzyme responsible for the reduction of oxidized glutathione (GSSG) to GSH **[176]**.

The level of reduced glutathione and the activities of glutathione-dependent enzymes were reduced significantly in rats administered with acetaminophen in the present investigation. The depression in GR contents along with GPx activity makes the cells more susceptible to toxic compounds. Administration of PCM for 21 days one dose every 48 h caused a reduction of GR and GPx levels in plasma. In contrast, administrations with either 100 µl of *E. elaterium* "fruit juice" or 300 mg/kg of vitamin C significantly prevented PCM-induced GR and GPx reduction, with greater effects demonstrated in 100 µl of *E. elaterium* "fruit juice" group.

Superoxide production comes from various sources such as the products of mitochondria. In human diseases superoxide is produced by the aerobic oxidation **[177]**.

The role of superoxide dismutase depletion in the pathogenesis of PCM intoxication. A decrease in SOD production can be attributed to an enhanced superoxide generation. The activity of SOD enzyme in plasma was also determined. The group treated with PCM showed significantly lower plasma SOD activity than the control group. Administration of 100 µl of *E. elaterium* "fruit juice" and vitamin C at 300 mg/kg significantly increased plasma SOD activity. As shown in pro/post- treatment groups.

CAT is a haemoprotein which is an important antioxidant enzyme. Catalase decomposes of H_2O_2 by dismutating it to form H_2O and O_2 **[178]**.

And protects tissue from reactive hydroxyl radicals is widely distributed in all animal tissues. Administration of PCM alone significantly reduced CAT activity, indicating oxidative stress. After administration of *E. elaterium* "fruit juice" the CAT level was increased significantly suggesting either the plant itself reacts with the reactive oxygen species or boosting the antioxidant enzyme production. Catalytic removal of reactive oxygen species by CAT enzyme is extremely important as it prevents numerous lipid peroxidation and organ dysfunction. Moreover, these finding support the beneficial effect of *Ecballium elaterium* "fruit juice" in maintaining the hepatocytes integrity and metabolic function. It is conceivable that these effects may be due, at least in part, to its higher antioxidant activity as a result to its phenolic content.

Peroxidation of acetaminophen to the semiquinone free radical would lead to redox cycling between the acetaminophen and the semiquinone **[179]**.

The free radicals attack the cell membrane, thus leading to destabilization and disintegration of the cell membrane as a result of lipid peroxidation **[180]**.

Lipid peroxidation has been postulated to be the destructive process in liver injury due to acetaminophen administration **[181]**.

In the present study, an elevation in the levels of MDA in the plasma of animals treated with acetaminophen was observed. The increase in MDA level suggest enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense

mechanisms to prevent formation of excessive free radicals. Administration of PCM induced oxidative stress with a significant increase in the levels of plasma MDA.

Concurrent administration of PCM and 100 µl of *E. elaterium* "fruit juice" and 300 mg/kg of vitamin C, reduced the oxidative stress, as evidenced by significantly reduced level of plasma MDA, than those in the PCM group. Interestingly, administration of both doses brought the MDA level nearly to the value of the control group as observed in the curative study.

The administration of silymarin showed an increase in the levels of GR and GPx in plasma, SOD and CAT, levels usually increases remarkably as an indicator in hepatocellular protection when compared with the acetaminophen treated group. The decrease observed in MDA level following the administration of silymarin may be attributed to the protection offered by the administration of the aqueous silymarin. The possible mechanism responsible for the protection of the acetaminophen induced liver damage by the silymarin may be as a result of its action as a free radical scavenger by intercepting the radicals involved in acetaminophen metabolism.

According to our results about the antioxidant enzymes "GR, GPx, SOD and CAT" in addition to the lipid peroxidation indicator "MDA". We may suggest that the therapeutic potential of *E. elaterium* "fruit juice" is dependent on an antioxidant mechanism. These results concluded that *E. elaterium* "fruit juice" inhibition PCM-induced tissue damage due to presence of various antioxidant bioactive compounds. The activity of vitamin C in our study has shown to be a potent antioxidant which mediates its antioxidant effect by scavenging free reactive oxygen species (ROS). Other studies have equally shown the protection of ascorbic acid in hepatic oxidative damage **[182, 183]**.

Thus, results of the present study suggests vitamin C's ameliorating effects to be likely mediated via inhibition of free radicals generation and/or free radical scavenging activity. Were we can notice these effects in prophylactic and curative groups when compared to the acetaminophen treated group "positive group".

The curative action of silymarin is associated with its antioxidant property, as it possibly acts as a free radical scavenger, an inhibitor of lipid peroxidation and a plasma membrane stabilizer **[168]**.

It acts as a preservative of liver GSH content and prevents lipid peroxidation. This effect may be due to its antioxidant activity. The protective effect of silymarin a free radical scavenger, may be due to an increase in the activity of antioxidant enzymes "superoxide dismutase and glutathione peroxidase" that in addition to the glutathione system, constitute the more important defense mechanism against damage by free radicals **[184]**.

PCM-induced renal insufficiency is consistent with acute tubular necrosis **[185]**. Oxidative stress is reported to play a role in the pathogenesis of PCM-induced renal damage **[186]**.

Plasma of blood urea and creatinine levels were significantly increased in groups treated with PCM alone, demonstrating that deterioration of the renal function, in comparison with those of the control and treated groups. Acetaminophen administration resulted in significant increase in the serum marker like blood urea and serum creatinine. makes it a candidate for its role in the pathogenesis induced renal damage.

However, supplementation with *E. elaterium* "fruit juice" silymarin at 50 mg/kg and 300 mg/kg of vitamin C significantly prevented further elevations of creatinine and blood urea, with obvious effects observed in silymarin group, as evidenced by no significant difference in plasma creatinine and blood urea level as compared to the control group.

Summary and conclusion

In the present study, *Ecballium elaterium* "fruit juice" possessed strong hepatoprotective and antioxidant activity in a rat model of acetaminophen-induced hepatotoxicity. The hepatoprotective activity of *Ecballium elaterium* "fruit juice" may be due to its free radical-scavenging and antioxidant activity, resulting from the presence of some phenolic compounds in the fruit juice of plant.

In general, to use this plant as safe prophylactic and curative agents, more studies should be carried out to know all the active components and their mechanism of actions weather synergistic or antagonist using different doses from this plant and another types of experimental animals for a long period in order to judgment if this plant could be use as safe agents or not in human therapy.

1. The prophylactic effect of different treatments against hepatotoxicity:

To study the protective effect of *Ecballium elaterium* "fruit juice" against hepatotoxicity, a total of 28 rats were used and the experiment lasted for 3 weeks. Animals were divided randomly into four groups each group contain 7 rats as follows:

Group 1: Rats were fed on the standard diet and served as negative control (-ve) for 3 weeks.

Group 2: Rats were administered with acetaminophen 400 mg/kg body weight orally every 48 h for 21 days; and served as positive control group (+ve).

Group 3: Rats were administered *Ecballium elaterium* "fruit juice" at a dose of 1ml/kg orally before 1 hour of oral administration of acetaminophen.

Group 4: Rats were administered vitamin C at a dose of 300 mg/kg body weight orally before 1 h of oral administration of acetaminophen.

2.4.2. The curative effect of different treatments against hepatotoxicity:

In this experiment, a total of 35 rats were used. 7 rats were fed on the standard diet and served as negative control (-ve) **group1**.

The other rats were subjected to the induction of experimental hepatotoxicity for 22 days where treated by acetaminophen at 400 mg/kg every 48 h and treated for 22 days.

The hepatotoxicity rats (28 rats) where divided randomly into equal 4 groups (each group contain 7 rats).

Group 2: Rats were served as hepatotoxicity animals (+ve).

Group 3: Rats were daily received *Ecballium elaterium* "fruit juice" at a dose of 1ml/kg orally for 22 days.

Group 4: Rats were daily received vitamin C at a dose of 300 mg/kg body weight orally for 22 days.

Group 5: Rats were daily received silymarin at a dose of 50 mg/kg body weight orally for 22 days.

blood samples were collected before and after induction of hepatotoxicity and then after administration of the different treatments.

The different biochemical parameters were carried out (serum transaminases, serum total lipids, Blood urea, serum creatinine, serum, serum alkaline phosphatase, serum lactate dehydrogenase, serum total protein and serum albumin) as well as the histopathological studies.

The results obtained revealed that *Ecballium elaterium* fruit juice has a prophylactic and curative effect against hepatotoxicity compared with the reference standard Silymarin in addition to vitamin C.

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الملخص العربي

نظرا للاستعمالات الشائعة للنباتات الطبية في علاج العديد من الأمراض وذالك لاحتواء هذه النباتات على قيمه غذائية عالية واحتوائها أيضا على العديد من المركبات الكيميائية ذات الفاعلية المضادة للأكسدة ولكونها مصادر طبيعية فإن ذلك يبشر بإمكانية استخدامها في علاج العديد من الأمراض والوقاية منها كبدائل للأدوية المخلقة كيميائياً.

وتهدف هذه الدراسة لتحديد الفاعلية المضادة للأكسدة لنبات قثاء الحمار وكذالك لمعرفة التأثير المضاد للتسمم الكبدي المستحدث باستعمال الباراسيتامول .

حيث تم تحديد الفاعلية المضادة للأكسدة من خلال تحديد المحتوي الكلي للمركبات الفينولية وكذالك الفلافونويد ومن خلال تثبط الجذر الحر لمركب (1, 1-diphenyl-2-picryl hydrazyl radical $DPPH^{\cdot}$) بالإضافة إلي القوة الاختزالية.

أما فيما يتعلق بإحداث التسمم الكبدي بالباراسيتامول فتم تحديده من خلال قياس القدرة التصنيعية و الإستخراجية للكبد بالإضافة إلي الإنزيمات المحددة لسلامة خلايا الكبد كذالك وقد تم قياس فاعلية الإنزيمات المضادة للأكسدة وذالك لتحديد مدى فاعلية النبات على هذه الإنزيمات.

وقد أجريت الدراسات البيولوجية على فئران التجارب البيضاء حيث تم تقسيمها إلي مجموعتين رئيسيتين:

أولا : دراسة التأثيرات الوقائية للنبات:

وفي هذه الدراسة استخدم عدد 28 فأر لمدة 3 أسابيع حيث تم سحب عينات الدم قبل بدء التجربة وتم السحب أيضا في نهاية التجربة. حيث تم توزيع هذه الفئران بالتساوي إلي أربع مجموعات (7 فئران في كل مجموعة).

المجموعة الأولى : استخدمت كمجموعة ضابطة.

المجوعة الثانية : عملت بالأسيتامينوفين "باراسيتامول" 400 ملجم/كجم.

المجموعة الثالثة :عملت بفيتامين سي 300 ملجم /كجم من وزن الفأر بالإضافة إلي بالأسيتامينوفين.

المجموعة الرابعة : عملت بالنبات بجرعة 1 مل/كجم بالإضافة إلي بالأسيتامينوفين.

ثانياً : دراسة التأثير العلاجية للنبات:

وفي هذه الدراسة استخدم عدد 35 فأر لمدة 6 أسابيع حيث تم سحب عينات الدم قبل بدء التجربة وبعد 3 أسابيع وفي نهاية التجربة. حيث تم توزيع هذه الفئران بالتساوي إلي خمس مجموعات (7 فئران في كل مجموعة). استخدم 7 فئران كمجموعة ضابطة " **المجموعة الأولى**" . بينما تم معالجة باقي المجاميع بالباراسيتامول 400 ملجم/كجم لمدة 3 أسابيع لإحداث التسمم الكبدي وبعد ذالك قسمت الفئران الي أربع مجاميع متساوية:

المجموعة الثانية : الإيجابيق التي تم معاملتها بالأسيتامينوفين "باراسيتامول"400 ملجم/كجم.

المجموعة الثالثة : عملت بالنبات 1 مل/كجم.

المجموعة الرابعة : عملت بفيتامين سي بجرعة300 ملجم/كجم من وزن الفأر.

المجموعة الخامسة : عملت بالسليمارين بجرعة50 ملجم/ كجم من وزن الفأر.

كذلك ثم اخذ عينات من الكبد بعد نهايق التجارب لعمل الدراسات الهستوباثولوجية.

أوضحت النتائج المتحصل عليها أن لهذا النبات فاعلية بيولوجيه على تقليل التسمم الكبدي الناتج من بالأسيتامينوفين "باراسيتامول" من خلال تحسين وظائف الكبد وذالك نظرا لمحتواها من مضادات الأكسدة كما أكد ذلك الدراسات الهستوباثولوجية.

وننصح بعدم التسرع في استخدام هذا النبات في علاج أمراض الكبد إلا بعد عمل المزيد من الدراسات لمعرفة ميكانيكية عمل المواد الفعالة بها واستخدام نوعيات مختلفة من حيوانات التجارب ولفترات أطول حتى يمكن تقييمها ومعرفة أثارها الجانبية لتحديد إمكانية استخدامها كعلاج للإنسان من عدمه.