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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *ILLICIUM VERUM* HOOK FRUITS IN RODENTS

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ABSTRACT

To evaluate the hepatoprotective activity of the ethyl acetate and methanolic extract of the fruits of Illicium verum against paracetamol and gentamicin induced hepatotoxicity using male Wistar rats. The plant Illicium verum consists of phytoconstituents like alkaloids, terpenoids, quercetin, flavonoids, glycosides, proteins, total phenolic compounds, sesquiterpines, saponins and tannins. The extract was safe up to 2000 mg/kg, so doses (200 and 400 mg/kg/bd. wt) of the methanolic and ethyl acetate extract of the fruits of Illicium verum were selected for the study. Liver functions were monitored via measuring serum and various tissue parameters like alanine transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALKP) and total bilirubin (BIT) and finally the tissues are subjected to histopathological studies. Paracetamol and gentamicin produced significant biochemical (increase in ALT, AST and ALKP) changes, histological (damage to hepatocytes) changes, induced by paracetamol and gentamicin in liver parameters. Treatment with Illicium verum extract significantly ($p<0.005$) prevented the physical, biochemical, and histological changes induced by paracetamol and gentamicin in the liver. Histopathological study revealed improvement in the architecture of hepatocytes. The ethyl acetate and methanolic extracts of Illicium verum showed moderate level of protection against paracetamol and gentamicin induced hepatotoxicity. Further studies are required to isolate the active constituents to establish the exact mechanism of action.

KEY WORDS: *Illicium verum, Hepatoprotective, Paracetamol, Gentamycin, Ethyl acetate, Methanol.*

INTRODUCTION

Many of the modern drugs mainly based on synthetic chemical compounds however have been found to have harmful side effects on human system. This has triggered off extensive research and development in the field of herbal medicine. In fact there is a growing demand for herbal medicine in most of the developed and developing countries of the world today. The causative factors of liver disorders include virus infection exposure to our consumption of certain chemicals. The substance that injures the liver cells in some people and results serious harm to the liver caused by drugs and by the combination of drugs and other substances is an important health problem [1].

Liver diseases are considered as fatal & life threatening. It creates a serious challenge to public health. Liver diseases are due to infection and / or exposure of liver to various toxic substances such as drugs or alcohol. Some times over dosage of drugs can also lead to liver damage. Now-a-day's due to inadequacy of liver protective agents, researchers and traditional medicine practioners concentrate in herbal based remedies for various liver disorders. Modern medicines have little to offer for alleviation of hepatic disorders. Treatment options for common liver diseases such as cirrhosis, fatty liver and chronic hepatitis are problematic. There was no safe hepatoprotective drug available for the treatment of liver disorders. There are few effective plants that cure liver diseases so considerable interest has developed in the examination of these numerous plants remedies which are useful in liver diseases.

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The present research is aimed at compiling the data on promising herbal extract from plant Illicium verum that have been tested in hepatotoxicity models.

Aminoglycosides, such as gentamicin, are a class of clinically important antibiotics used extensively in the treatment of infections, particularly against aerobic gram-negative bacteria. Despite their beneficial effects, aminoglycosides have considerable nephrotoxic side effects [3] Gentamicin has been used clinically due to its wide spectrum of activities against Gram-negative bacterial infections caused by Pseudomonas, Proteus and Serratia [4]. In many cases, gentamicin is the only effective therapeutic drug against bacterial strains resistant to other antibiotics, but nephrotoxic side effects limit its use. Gentamicin is well recognized to produce renal tubular necrosis mainly in the proximal tubules. This drug causes generation of reactive oxygen species (ROS) and nitrogen species, which induce cellular injuries and necrosis via decrease in the activity level of antioxidant enzymes and increase of lipid peroxidation of renal samples [5]. It is one of the aminoglycosides that also causes liver toxicity. Most of the intravenously administered dose is excreted in the urine, whereas some of the aminoglycoside injected is selectively accumulated in the renal cortex leading to renal cell injury [6]. Gentamicin toxicity occurs in about 15–30% of treated subjects, is manifested clinically as nonoligouric renal failure, with a slow rise in serum creatinine that develops after several days of treatment. The drug is nephrotoxic because a small but sizable portion (about 5%) of the administered dose is retained in the epithelial cells lining in certain segments of the proximal renal tubules [7]. Approximately, 5–10% of the patients treated with aminoglycosides experience such toxic effects. The association of aminoglycosides with negatively-charged phospholipids and their accumulation in the lysosomes of tubular cells leads to phospholipidosis by inhibition of lysosomal phospholipases and trigger necrosis [8]. It has been reported that at cellular level, aminoglycosides interfere with protein synthesis [9]. Although the main cause of gentamicin induced nephrotoxicity is unknown, however, generation of ROS has been monitored during in vitro and in vivo studies. Gentamicin acts as iron chelator and complexes

of iron-gentamicin are considered potent catalysts for Fenton reaction [10]. Damages induced with oxidative stress to renal cortex mitochondria releases iron, and represent one of several mechanisms to trigger the production of free radicals. Other sources of renal damages might include the vasoconstriction induced with platelet activation factor and thus decrease blood flow and glomerular filtration rate [11]. Gentamicin may bind to phospholipids of intracellular and extracellular membranes to alter the function and/or causes damages [12, 13]. Treatment of gentamicin causes depletion of ATP either through mitochondrial damage and/or inhibition of mitochondrial oxidative phosphorylation [14].

Paracetamol is a drug of para-aminophenol group which is considered one of the commonly used and safe over the counter antipyretic and analgesic drugs, when administered at recommended doses [15]. The main problem with this medication remains its misuse through intentional or unintentional ingestion of supra- therapeutic dosages which usually lead to hepatic necrosis [16]. Oxidative stress is reported to constitute a major mechanism in the pathogenesis of Paracetamol induced liver and renal damage in experimental animals. Because toxic overdoses of Paracetamol were reported to have life-threatening impacts on the liver and kidney, e.g. hepatic necrosis and renal failure in both human and experimental animals, early protection from Paracetamol induced nephrotoxicity has life-saving importance. Therefore, supplementation with antioxidants is very crucial to delay, prevent or remove oxidative damage [17].

MATERIALS AND METHODS

Plant material:

The plant Illicium verum hook fruits was collected from Hyderabad, Ranga reddy district, Telangana state in the month of December 2016, this material was identified and authenticated by pharmacognist or botanist.

Preparation of plant extract:

The freshly dried hook fruits of the plant Illicium verum were collected which was already shade dried and they were pulverized in the laboratory.

Soxhlet extraction:

Soxhlet extraction is the process of continuous extraction in which the same solvent can be circulated through the extractor for several times. This process involves extraction followed by evaporation of the solvent. The vapors of the solvent are taken to a condenser and the condensed liquid is returned to the drug for continuous extraction. 500 gm of Illicium verum hook fruits powder was used for the extraction with ethyl acetate and methanol. The extract was collected and yield was calculated.

Acute toxicity testing:

Female rats were used for this purpose. The animals were fasted overnight, providing only water, after which the extract was administered to the respective groups orally at the dose level of 2000 mg/kg bd. wt by gastric intubation and the groups were observed continuously for 24 hr for behavioral, neurological and autonomic profiles, and then at 24 hr and 72 hr for any lethality. The animals were further observed for toxic symptoms for 14 days. According to the OECD 425 guidelines if mortality is observed in 2 or 3 animals, then the dose administered is assigned as a toxic dose. If mortality is observed in one animal, then the same dose is repeated again to confirm the toxic dose. If mortality is not observed at all, the plant extract is considered as non-toxic.

Methods for evaluation of Hepatoprotective activity:

1. Paracetamol induced hepatotoxicity model in rats
2. Gentamicin induced hepatotoxicity model in rats

1. Paracetamol induced hepatotoxicity model in rats:

Paracetamol toxicity is caused by excessive use or overdose of the medication paracetamol (acetaminophen). Drug-induced liver damage (hepatotoxicity) results not from paracetamol itself, but from one of its metabolites, N-acetyl-p-benzoquinoneimine (NAPQI). NAPQI decreases the liver's natural antioxidant glutathione and directly damages cells in the liver, leading to liver failure [18].

Study protocol:

A total of 42 animals were taken and were divided into 7 groups of 6 animals each (n=6 / group). Group I (control) received normal saline orally for 7 days. Group II (Disease control) received a Paracetamol at a dose of 500 mg/kg, bd. wt p.o for 7 days. Group III & Group IV (Test) were administered with ethyl acetate extract Illicium verum hook fruits at a dose of 200 and 400 mg/kg, bd. wt p.o from 4th day to 7th along with Paracetamol at a dose of 500 mg/kg, bd. wt p.o. for 7 days. Group V & VI animals were administered with methanolic extract of Illicium verum hook fruits at a dose of 200 & 400 mg/kg, bd. wt p.o. from 4th day to 7th followed by Paracetamol at a dose of 500 mg/kg, bd. wt p.o. Group VII animals were treated with Standard hepatoprotective drug Silymarin from 4th day to 7th at a dose of 250 mg/kg, bd. wt p.o followed by Paracetamol - 500 mg/kg, bd. wt i.p. for 7 days. On 8th day, the animals were anaesthetized using isoflurane anaesthesia and blood was collected by retro-orbital plexus. Serum was separated by centrifugation of blood at 3,000 rpm for 10 min and the separated serum was used for further biochemical analysis and kidney and liver tissues were isolated and subjected for histopathological studies [18].

2. Gentamicin induced hepatotoxicity model in rats:

Gentamicin is well recognized to produce renal tubular necrosis mainly in the proximal tubules. This drug causes generation of reactive oxygen species (ROS) and nitrogen species, which induce cellular injuries and necrosis via decrease in the activity level of antioxidant enzymes and increase of lipid peroxidation of renal samples. It is one of the aminoglycosides that also causes liver toxicity. Most of the intravenously administered dose is excreted in the urine, whereas some of the aminoglycoside injected is selectively accumulated in the renal cortex leading to renal cell injury.

Study protocol:

A total of 42 animals were taken and were divided into 7 groups of 6 animals each (n=6 / group). Group I (control) received normal saline orally for 21 days. Group II (Disease control) received a gentamicin at a dose of 80 mg/kg, bd. wt i.p. for 21 days. Group III & Group IV (Test) were administered with ethyl acetate extract Illicium verum hook fruits at a dose of 200 and 400 mg/kg, bd. wt p.o. from 9th day to 21st along with gentamicin at a dose of 80 mg/kg, bd. wt i.p. for 21 days. Group V & VI animals were administered with methanolic extract of Illicium verum hook fruits at a dose of 200 & 400 mg/kg, bd. wt p.o. from 9th day to 21st followed by gentamicin at a dose of 80 mg/kg, bd. wt i.p. Group VII animals were treated with Standard hepatoprotective drug Silymarin from 4th day to 7th at a dose of 100 mg/kg, bd. wt p.o followed by gentamicin at a dose of 80 mg/kg, bd. wt i.p. for 21 days. On 22nd day, the animals were anaesthetized using isoflurane anaesthesia and blood was collected by retro-orbital plexus. Serum was separated by centrifugation of blood at 3,000 rpm for 10 min and the separated serum was used for further biochemical analysis and kidney and liver tissues were isolated and subjected for histopathological studies [19].

RESULTS & DISCUSSIONS

Percentage yield of extract:

The % yield of the extracts was calculated and found to be 8.3 g of methanolic and 9.1 g of ethyl acetate extract.

Preliminary phytochemical screening:

Preliminary phytochemical analysis of Illicium verum fruit extracts was performed.

Table No. 1: Report of preliminary phytochemical screening

S. No	Constituents	Methanolic extract	Ethyl acetate extract
1	Alkaloids	+++	-
2	Terpenoids	+	+++
3	Phenols	+	++
4	Glycosides	++	+
5	Flavonoids	+	++
6	Proteins and Amino acids	---	---
7	Sterols	+	++
8	Carbohydrates	+	+
9	Tannins	++	---
10	Saponins	++	---

'+' = present, '-' = absent

Gentamicin induced hepatotoxicity:

In gentamicin induced hepatotoxicity the activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALKP) and total bilirubin level showed a significant ($p<0.05$) increase in gentamicin treated animals as compared to control group. Administering of EAIV (200 & 400 mg/kg, bd. wt *p.o*) and MEIV (200 & 400 mg/kg, bd. wt *p.o*) significantly reduced the levels of AST, ALT, ALKP and total bilirubin level in gentamicin treated rats as compared to the group treated with gentamicin alone (Table No.2).

Table No. 2: Effect of *Illicium verum* extracts in gentamicin induced hepatotoxicity

S. No.	Groups	ALT	AST	ALP	Bilirubin
1	Normal Control	19.75 ± 0.0735	32.66 ± 0.4443	24.46 ± 0.4970	1.6 ± 0.032
2	Disease Control	30.533 ± 0.3160 b,A	65.65 ± 0.0442 b,A	55.716 ± 0.0970 b,A	15.416 ± 0.0435 b,A
3	EAIV 200 mg/kg, bd. wt	23.6 ± 0.0375 b,**,A	24.43 ± 0.5488 b,**,A	26.466 ± 0.5767 b,**,A	0.590 ± 0.1058 b,**,A
4	EAIV 400 mg/kg, bd. wt	15.10 ± 0.8737 a,**,A	22.316 ± 0.326 b,**,A	35.5196 ± 0.2673 b,**,B	0.62 ± 0.106 b,**,B
5	MEIV 200 mg/kg, bd. wt	28.833 ± 0.3364 b,**,A	24.833 ± 0.012 b,**,A	26.580 ± 0.4749 b,**,A	0.560 ± 0.0876 b,**,B
6	MEIV 400 mg/kg, bd. wt	23.60 ± 0.0806 b,**,A	21.63 ± 0.1077 b,**,A	31.566 ± 0.3109 b,**,B	0.6 ± 0.0632 b,**,B
7	Standard (Silymarin) 100 mg/kg, bd. wt	17.33 ± 0.0480 b,**	27.633 ± 0.262 b,**	28.30 ± 0.337 b,**	0.91 ± 0.0389 b,**

*Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group, disease control group and standard group. Significant values are expressed as control group (a=p<0.01, b=p<0.05), disease control group (**= p<0.01, *= p<0.05) and standard (A = p < 0.01, B = p < 0.05), ns- non significant.*

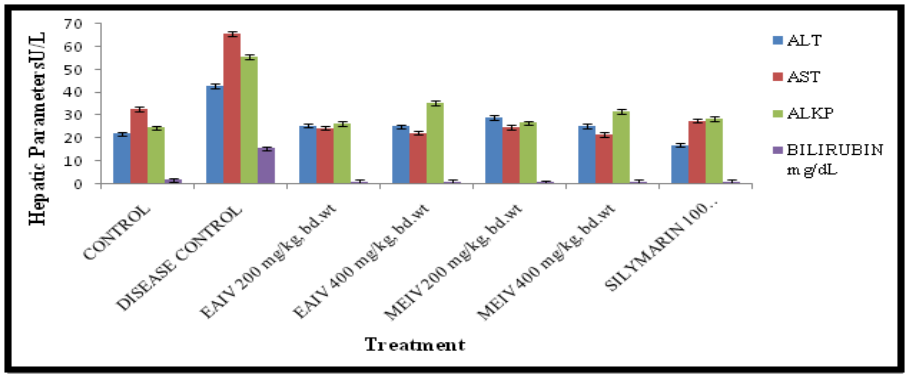


Fig. 1: Effect of *Illicium verum* fruit extracts on gentamicin induced hepatotoxicity

The present study examined the hepatoprotective effects of *Illicium verum* hook fruits methanolic & ethyl acetate extracts against gentamicin-induced hepatotoxicity in rodents.

Gentamicin has also been shown to induce hepatotoxicity secondary to nephrotoxicity and ototoxicity. It induces hepatic injury by promoting the generation of free radicals which attack and destroy hepatocytes (Noorani *et al.*, 2010; Al-Kenanny *et al.*, 2012). Drug induced hepatotoxicity is characterized by elevated levels of ALT, AST, ALP, GGT and bilirubin as well as destruction of tissue histology (Kurtovic and Riordan, 2003; Sivakrishnan and Kottaimuthu, 2013; Adejuwon *et al.*, 2014). High levels of these enzymes are consequences of hepatocytes destruction and increase in cellular permeability (Sivakrishnan and Kottaimuthu, 2013), whereas increased amount of bilirubin is indicative of loss of functional efficiency of hepatic cells in binding, conjugating and excreting bilirubin (Saroswat *et al.*, 1993; Singh *et al*., 2005).

In this study, gentamicin showed significant increase in the levels of liver biomarkers (ALT, AST, ALKP, and bilirubin) and decrease levels of total proteins, globulin and albumin as well as cause severe liver destruction. However, administration of extract significantly normalized the levels of these markers and restored liver structure. This finding correlates with previous reports by Noorani *et al.,* (2010) and Al-Kenannay *et al.,* (2012), who also showed that gentamicin induces hepatotoxicity evidenced by elevated levels of serum enzymes. Methanolic and ethyl acetate extracts of *Illicium verum* significantly reduced the damage caused by gentamicin probably through a protective effect against cellular destruction and restoration of hepatocytes integrity.

Effect of extracts on antioxidant parameters in gentamicin induced hepatotoxicity:

Antioxidant activity of the liver was studied to assess the effect of *Illicium verum* in ameliorating the oxidative stress. The mean ± SEM values of SOD, GSH, CAT and LPO in the liver tissue are shown in Table No.3.

Table No. 3: Effect of extracts on Antioxidant parameters in gentamicin induced hepatotoxicity

GROUPS	TREATMENT	SOD Levels (mg/protein)	GSH Levels (µg/mg)	CAT Levels (µM/min/mg)	LPO Levels (nm/mg)
1	Normal Control	0.78±0.017	0.94±0.017	7.94±0.2	5.94 ± 0.09
2	Gentamicin 80 mg/kg, bd. Wt	0.30±0.005 a,A	0.54±0.01 a,B	1.84±0.04 a,A	14.14 ± 0.09 a,A
3	EAIV 200 mg/kg, bd. Wt	0.44±0.02 a,**,A	0.63±0.01 a,**,B	3.23±0.07 a,**,A	3.43 ± 0.07 a,**,A
4	EAIV 400 mg/kg, bd. Wt	0.61±0.01 a, **,A	0.78±0.01 a,**, B	5.18±0.05 a,**, A	5.18 ± 0.06 a,**, A
5	MEIV 200 mg/kg, bd. Wt	0.48±0.02 a,**,A	0.73±0.01 a,**,B	3.33±0.07 a,**,A	4.43 ± 0.07 a,**,A
6	MEIV 400 mg/kg, bd. Wt	0.59 ± 0.01 a, **,A	0.78 ± 0.01 a,**, B	5.18 ± 0.05 a,**, A	6.18 ± 0.06 a,**, A
7	Silymarin 100 mg/kg, bd. wt)	0.73 ± 0.01 a,**	0.83 ± 0.017 a,**	1.84 ± 0.04 a,**	5.83 ± 0.17 a,**

*Values are expressed as Mean±SEM (n=6). All the groups were compared with control group and standard group (Dunnet's t test). Significant values are expressed as control (a = p<0.001, b= p<0.05) gentamicin control (** = p<0.001, * = p<0.05) standard (A = p<0.01, B = p<0.05).*

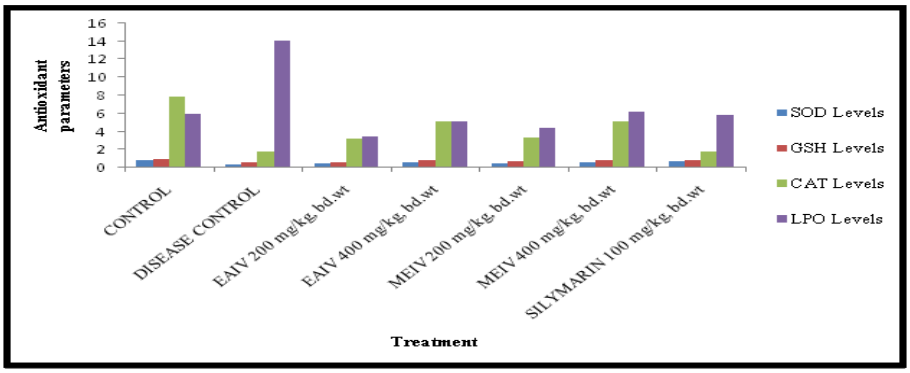


Fig. 2: Effect of *Illicium verum* fruit extracts on anti-oxidant parameters

There was a significant (p<0.05) increase in liver LPO activity in the gentamicin treated group when compared with the control group. *Illicium verum* at low dose level (200 mg/kg b.w.) significantly reduced the LPO activity when compared with the high dose (400 mg/kg b.w.) group. Gentamicin administered group showed significant (p<0.05) reduction of SOD activity when compared to the control group. On the other hand, the SOD activity of *Illicium verum* treated group was similar to that of the control group and was also comparable to that of Silymarin group (Table No. 3).

There was a significant (p<0.05) decrease in the hepatic CAT activity in the gentamicin treated group when compared to the control group. Dose dependent significant (p<0.05) increase in CAT activity was observed in the plant extract treated groups. Silymarin also produced a similar effect (Table 2). There was a significant (p<0.05) reduction in the liver GSH content in the gentamicin group when compared to the control group. Even though *Illicium verum* at the lower dose significantly increased the GSH content when compared to gentamicin group, they were unable to restore the liver GSH content back to that of the control level. Significant (p<0.05) restoration of GSH content towards the control levels were observed in the higher dose group of *Illicium verum* which was also comparable to that of Silymarin (Table No. 3).

The free radicals generated by the administration of gentamicin initiate not only the peroxidation of polyunsaturated fatty acids in the cell membrane, but also covalently bind to microsomal lipids and proteins. (Manna *et al*., 2006) This phenomenon results in the generation of reactive oxygen species (ROS) such as the superoxide anion, H_2O_2 and OH. Various enzymatic and non-enzymatic pathways are utilized by the cell to cope up with the ROS and other free radicals. However, once a condition of oxidative stress is established in the cell, it has been reported that SOD, CAT and GST constitute a mutually supportive team of defence against ROS. The decreased activity of SOD in the liver of gentamicin treated animals may be due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. Increased utilization of GSH by the free radicals generated due to gentamicin toxicity might lead to a decreased GSH content (Manna et al., 2006).

Paracetamol induced hepatotoxicity:

Hepatic toxicity is reflected by increase in the biochemical parameter levels such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), and total bilirubin. Treatment of rats with paracetamol resulted in severe damage of hepatocytes, biliary obstruction and transport inability across the liver as indicated by high levels of AST, ALT, ALP and bilirubin (Edwards and Bouchier, 1991). Pre-treatment of rats with Silymarin, significantly (P < 0.001) decreased the raised levels of AST, ALT, ALP and bilirubin induced by paracetamol indicating a good recovery from the hepatotoxic agent. The hepatoprotective effect offered by EAIV & MEIV at 200 mg/kg, bd. wt & 400 mg/kg, bd. wt doses was found to be highly significant (p < 0.001) in all parameters studied with reduction in AST, ALT, ALP and bilirubin respectively (Table No. 4).

Table No. 4: Effect of *Illicium verum* extracts in paracetamol induced hepatotoxicity

S. No.	Groups	ALT	AST	ALKP	Bilirubin
1	Normal Control	15.83 ± 0.365	21.66 ± 0.3409	24.46 ± 0.4157	0.683 ± 0.0301
2	Disease Control	38.65 ± 0.5539 b, A	55.46 ± 0.0957 b,A	54.91 ± 0.0992 b,A	9.63 ± 0.0544 b,A
3	EAIV 200 mg/kg, bd. wt	26.483 ± 0.5595 b,**,A	29.33 ± 0.1378 b,**,A	32.366 ± 0.524 b,**,B	2.290 ± 0.102 b,**,A
4	EAIV 400 mg/kg, bd. wt	24.483 ± 0.1190 b,**,A	25.71 ± 0.2202 b,**,A	28.066 ± 1.2641 b,**,A	1.260 ± 0.108 b,**,A
5	MEIV 200 mg/kg, bd. wt	25.28 ± 0.3208 b,**,A	39.80 ± 0.1482 b,**,A	35.2 ± 0.527 b,**,A	4.416 ± 0.0881 b,**,A
6	MEIV 400 mg/kg, bd. wt	23.383 ± 0.4492 b,**,A	26.966 ± 0.080 b,**,A	32.166 ± 0.324 b,**,A	2.59 ± 0.0621 b,**,A
7	Standard(Silymarin) 100 mg/kg, bd. wt	21.33 ± 0.7808 b,**	28.2 ± 0.1087 b,**	30.7 ± 0.311 b,**	1.9000 ± 0.0377 b,**

*Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group, disease control group and standard group. Significant values are expressed as control group (a=p<0.01, b=p<0.05), disease control group (**= p<0.01, *= p<0.05) and standard (A = p < 0.01, B = p < 0.05), ns- non significant.*

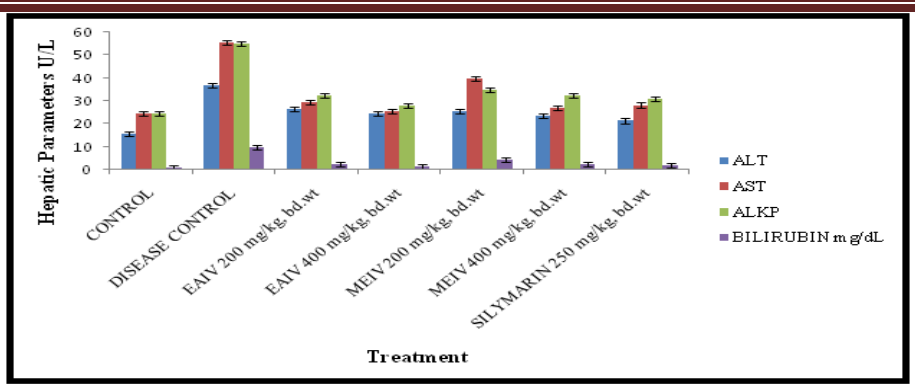


Fig. 3: Effect of *Illicium verum* fruit extracts on paracetamol induced hepatotoxicity

Effect of *Illicium verum* extracts in paracetamol induced hepatotoxicity:

Therapeutic doses of paracetamol eliminated mainly as sulfate and glucoronide conjugates (Eriksson *et al*., 1992) and only 5 % of the dose is converted into N-acetyl-p-benzoquineimine (NAPQI). However, upon administration of toxic doses of paracetamol, higher percentages of the molecules are oxidized to highly reactive NAPQI by cytochrome P450 enzymes. Semiquinone radicals, obtained by one electron reduction of NAPQI are rapidly conjugated with glutathione (GSH), a sulphydryl donor which results in the depletion of liver GSH (glutathione) pool (Remirez *et al*., 1995). Under conditions of excessive NAPQI formation or reduced of glutathione store, NAPQI covalently binds to vital proteins, the lipid bilayer of hepatocyte membranes and increases the lipid peroxidation (McConnachie *et al*., 2007).

The serum levels of AST, ALT, ALP and bilirubin in the groups treated with EAIV & MEIV at 200 mg/kg, bd. wt & 400 mg/kg, bd. wt showed moderate decreases in all measured parameters. Results were found to be statistically significant. EAIV and MEIV showed effect on the level of AST, ALT, ALP and bilirubin. Animal treated with this extracts of *Illicium verum* showed good significant reduction in the parameters studied (Table No. 3). However, highly significant ($p<0.001$) reduction in hepatic parameters level was observed in animal treated with paracetamol.

Table No. 5: Effect of extracts on Antioxidant parameters in paracetamol induced hepatotoxicity

Groups	Treatment	SOD Levels (mg/protein)	GSH Levels (µg/mg)	CAT Levels (µM/min/mg)	LPO Levels (nm/mg)
1	Normal Control	0.54 ± 0.012	9.04 ± 0.017	0.934 ± 0.017	11.04 ± 0.09
2	Paracetamol 500 mg/kg, bd. wt	0.27 ± 0.08 a,B	5.27 ± 0.01 a,A	0.108 ± 0.01 a,A	14.54 ± 0.09 a,A
3	EAIV 200 mg/kg, bd. wt	0.37 ± 0.01a,*,B	6.57 ± 0.019 a,*,A	0.46 ± 0.019 a,*,A	7.03 ± 0.07 a,**,A
4	EAIV 400 mg/kg, bd. wt	0.46 ± 0.06 a, *, B	8.76 ± 0.018 a, *, A	0.52 ± 0.018 a, *, A	5.48 ± 0.06 a,**, A
5	MEIV 200 mg/kg, bd. wt	0.39 ± 0.01 a,*,B	7.57 ± 0.016 a,*,A	0.49 ± 0.019 a,*,A	7.09 ± 0.09 a,**,A
6	MEIV 400 mg/kg, bd. wt	0.39 ± 0.06 a, *, B	8.96 ± 0.018 a, *, A	0.49 ± 0.018 a, *, A	6.5 ± 0.05 a,**, A
7	Silymarin 250 mg/kg, bd. wt	0.54 ± 0.02 a,*	7.24 ± 0.026 a,*	0.74 ± 0.026 a,*	9.03 ± 0.17 a,**

*Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group, diseased control group and standard group. Significant values are expressed as control group (a=p<0.01, b=p<0.05), paracetamol control group (**= p<0.01, *= p<0.05) and standard (A = p < 0.01, B = p < 0.05), ns- non significant.*

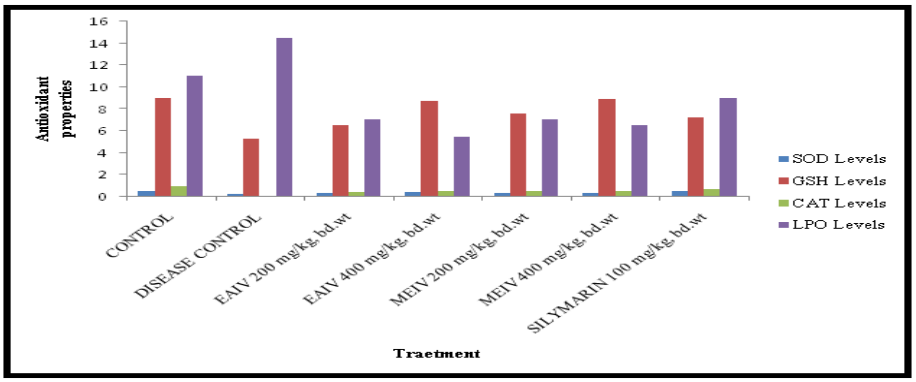


Fig. 4: Effect of *Illicium verum* fruit extracts on Anti-oxidant parameters

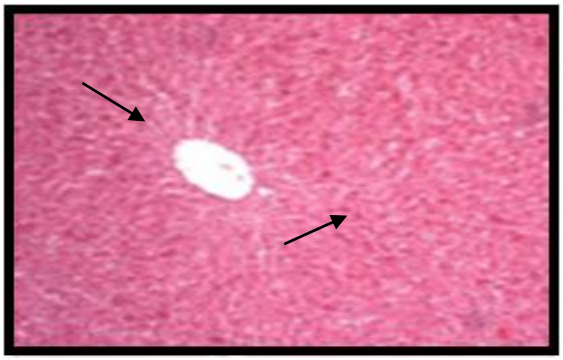
Lipid peroxidation has been postulated to be the destructive process of liver injury due to paracetamol administration [Muriel *et al*., 1992]. In the present study, elevations in the levels of end products of lipid peroxidation in liver of rats, treated with paracetamol, were observed. The increase in MDA level in liver suggested enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms to prevent formation of excessive free radicals. Treatment with extracts of *Illicium verum* significantly and dose dependently reversed these changes. Hence, it may be postulated that, the hepatoprotective action of the extracts was due to their antioxidant effect. Here also, the hepatoprotective effects of *Illicium verum* were found to be better. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defence system. It scavenges the superoxide anion to form hydrogen peroxide [Curtis *et al*., 1972; Korsrud *et al*., 1973]. In the present study, it was observed that extracts caused a significant and dose dependent increase in the hepatic SOD activity of the paracetamol intoxicated rats. Catalase is an enzymatic antioxidant widely distributed in all animal tissues and the highest

activity is found in the red cells and in liver. Catalase decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals [Chance *et al.,* 1952]. In the present study, it was observed that, the extracts caused a significant and dose dependent increase in the hepatic catalase activity of the paracetamol intoxicated rats.

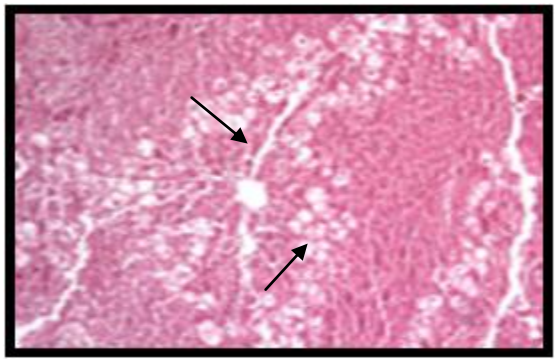
Histopathological study:

Histopathological study of liver in gentamicin and paracetamol induced hepatotoxicity:

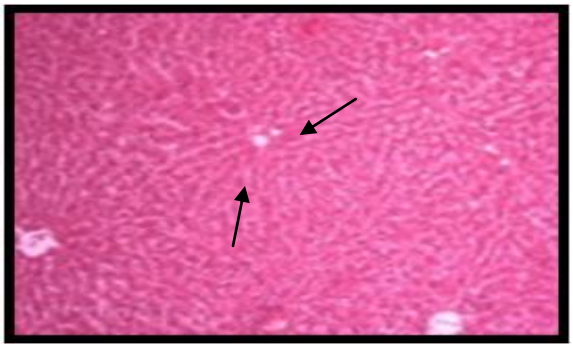
- The histological appearance of the hepatocyte reflects their conditions (Prophet et al., 1994). Extracts that gave good protection in the biochemical parameters were subjected to the histopathological study as well. Liver cells as well. Liver cells of rats treated with 80 mg/kg bd. wt & 500 mg/kg bd. wt of gentamicin & paracetamol (Fig.No:4.7 A & Fig. No: 4.8 A) showed great damage represented by extensive focal necrosis, lymphocytic infiltrate, extensive hydrophic swelling with rosette formation, lymphocytic exudates and dilated congested vessels in portal tracts.
- Histopathological appearance of liver cells obtained from subgroup treatment with 200 mg/kg bd. wt EAIV before intoxication by gentamicin & paracetamol (Fig. No: 475 C & Fig. No: 4.8 C) showed the best degree of protection obtained in the current study with normal lobules, mild central focal necrosis, mild congestion in central veins and mild infiltration in portal tracts.
- Subgroup treated with 400 mg/kg bd. wt EAIV (Fig.No:4.7 D & Fig. No: 4.8 D) showed mild infiltrated congested portal tracts, dilated congested central vein, dilated congested sinusoids and focal necrosis.
- Administration of 200 mg/kg bd. wt MEIV (Fig. No: 4.7 E & Fig. No: 4.8 E) showed moderate protection represented by normal lobule, moderate portal tract dilation, congestion and central vein congestion.
- Administration of 400 mg/kg bd. wt MEIV (Fig. No: 4.7 F & Fig. No: 4.8 F) architecture, The hepatocytes are degenerating and appear to have dropped out, numerous vacuoles are seen nuclei of varying shapes and sizes mostly pycnotic are seen, nucleoli are absent and clumping of nuclear material is evident, there is also loss of normal sinusoidal space and rupture of the central vein.
- Liver cells treated with 250 mg/kg bd. wt of the standard drug Silymarin (Fig.No:4.7 G & Fig. No: 4.8 G) prior to paracetamol administration showed improvement in the liver cell histopathology with granular cytoplasm, mild congestion in central veins, mild portal tract infiltration and few focal necrosis.



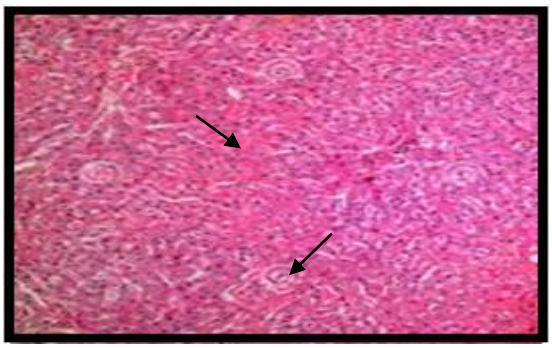
A. Normal



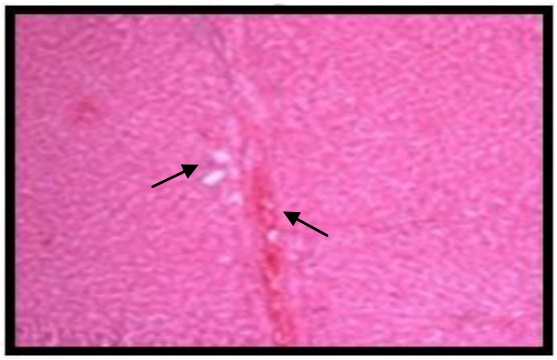
B. Disease control



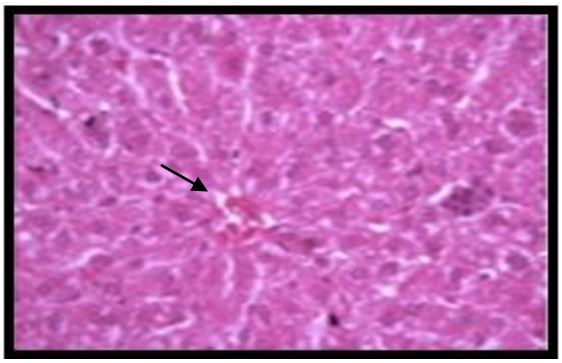
C. EAIV 200mg



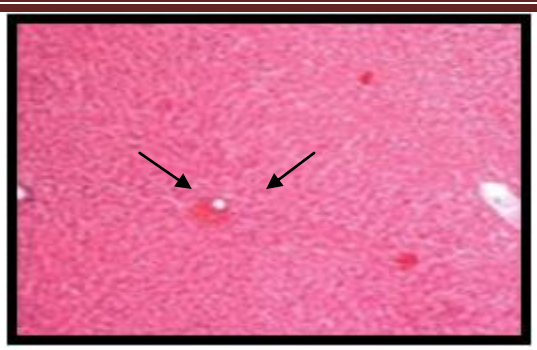
D. MEIV 400mg



E. EAIV 200 mg

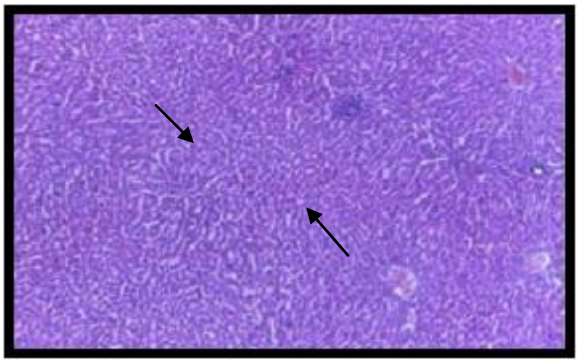


F.MEIV 400 mg

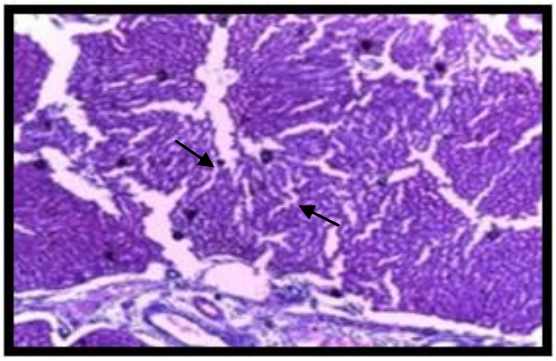


G. Standard Silymarin 100 mg

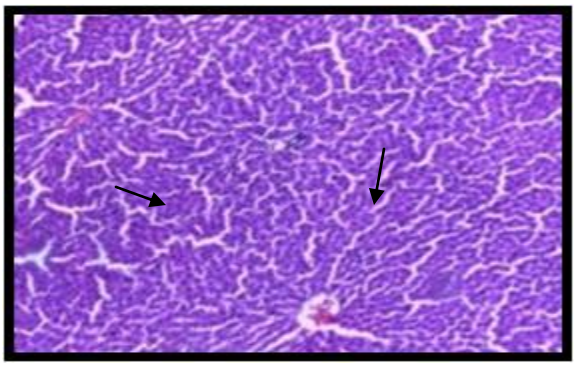
Fig. 5: Histopathology of the liver tissues (Gentamicin induced hepatotoxicity)



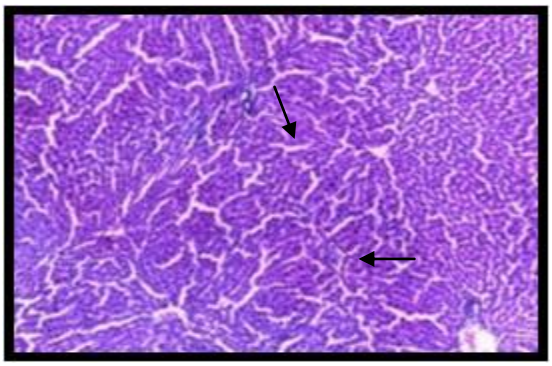
A. Control



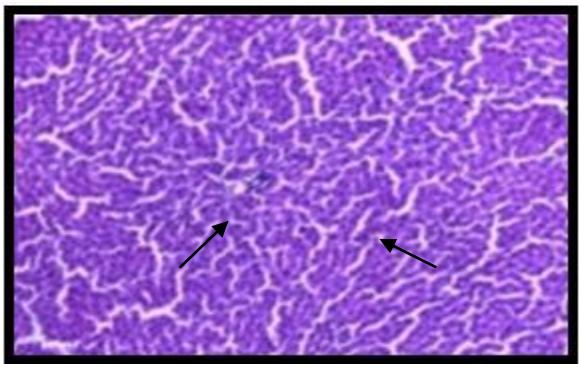
B. Disease control



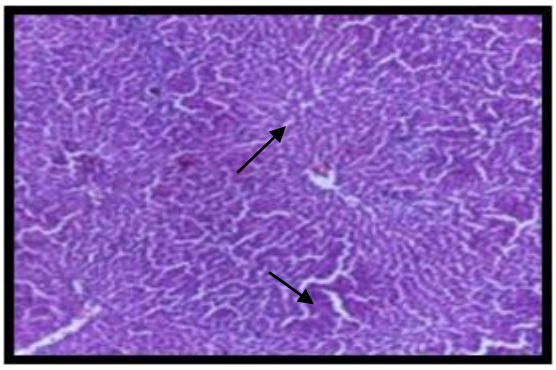
C. EAIV 200 mg



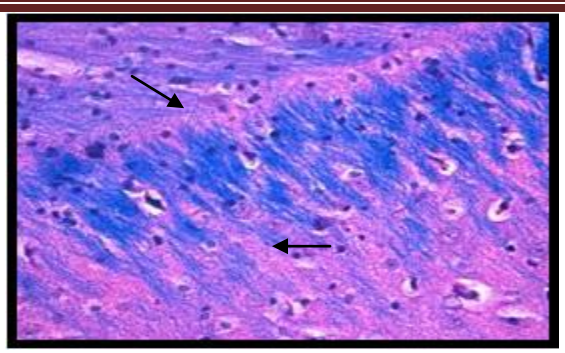
D. EAIV 400 mg



E. MEIV 200 mg



F. MEIV 400 mg



G. Standard (silymarin 250 mg)

Fig. 6: Histopathology of the liver tissues (Paracetamol induced hepatotoxicity)

CONCLUSIONS

The protective effect of these plants' extract against gentamicin and paracetamol may be related to polyphenolic compounds, terpenoids, alkaloids, phytosterols, *etc.* Polyphenolic compounds such as flavonoids can protect the cells against emptying reduced glutathione via increasing the capability of antioxidant enzymes (such as CAT, SOD and glutathione peroxidase). Flavonoids, which act as antioxidant, free radical scavenging and antilipoperoxidant agents, are helpful for hepatoprotection. Furthermore, these compounds with antioxidant properties can counteract free radicals in the environment and therefore avoid their destructive effects. However further studies are required to evaluate and standardize the constituents which are responsible for the hepatoprotective activity and to confirm their exact mechanism of action.

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