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Research Article

THE PROTECTIVE POTENTIAL EFFECTS OF *ATROPA ACUMINATA* ON ACETAMINOPHEN INDUCED HEPATOTOXICITY AND OXIDATIVE STRESS IN MALE ALBINO RATS

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ABSTRACT

Atropa acuminata *(*AC) is a traditional medicinal plant that is commonly used for disorders like , Central nervous system abnormalities, and in ayurvedic medicine, it is used for the treatment of fevers, chicken pox, colds, colitis, conjunctivitis (inflamed eyes) and diarrhea. This plant extract has also been reported for its varied biological activites such as antispasmodic, anxiety, arthritis, asthma, bedwetting and bowel disorders.

The main constituents of AC were found belonging to monoterpene, sesquiterpene, phenylpropanoid, flavonoid and quinone. As a traditional herbal medicine, *Atropa acuminata* was studied for various biological activities. However , the antihepatotoxic effect of this plant extract has not been shown in scientific research work .Having this in view the present study is aimed to evaluate the antihepatotoxic and antioxidant activities of ethanolic extract of *Atropa Acuminata* at two dose level of 250mg/kg & 500 mg/kg B/W on acetaminophen induced hepatotoxicity in rats. It was observed that the ethanol extract of AC conferred significant hepatoprotectivity. Biochemical and histopathological observations confirmed the beneficial roles of ethanol extract of AC against acetaminophen induced liver injury in rats. The activity of ethanol extract of AC (750 mg/kg B/W) was comparable to the standard drug silymarin (25mg/kg B/W).

Keywords: Hepatoprotective, *Atropa acuminata*, Acetaminophen, Silymarin, Antioxidant.

INTRODUCTION

Atropa acuminata is a folklore Indian medicinal plant .It is commonly known as Indian belladonna that can be cultivated in India and has established demand for their raw materials[1].All parts of the plant contain the alkaloids, atropine, hyoscyamine and bellodonnine, which are used as a sedative, antispasmodic, in convulsive disorders and as an antidote for poisoning. This medicinal plant also proved to be effective in homeopathic treatment for skin reactions during radiotherapy treatment for breast cancer[2].

The aerial parts are used to treat abnormal menstrual periods, acute infections, acute inflammation, anesthetic, antispasmodic, anxiety, arthritis, asthma, bedwetting, bowel disorders, chicken pox, colds, colitis, conjunctivitis (inflamed eyes), dental conditions, diarrhea,[3,4]diuretic (use as a "water pill"), diverticulitis, earache, encephalitis (inflammation of the brain), eye disorders (dilation of the pupils), fever,[5,6&7] flu, glaucoma, gout, hay fever, hemorrhoids, hyperkinesis (excessive motor function), inflammation, kidney stones, measles, motion sickness, mumps, muscle and joint pain, muscle spasms (excessive unintentional muscle movements), nausea and vomiting during pregnancy, organophosphate poisoning, pain from nerve disorders, parkinson's disease, pancreatitis, peritonitis, rash, scarlet fever, sciatica, [8,9] (back and leg pain), sedative,[10,11] sore throat, stomach ulcers, teething, toothache, ulcerative colitis,[12,13] urinary tract disorders (difficulty passing urine), warts, whooping cough[14,15]. However, no datas are available on the hepatoprotective and antioxidant properties of this plant. Therefore, this study was designed to investigate the protective effects of the ethanol extract of *Atropa acuminata* against acetaminophen (APAP)-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant Material

Aerial part of Atropa *acuminata* (Solanaceae) was collected from Tirunelveli district, Tamil Nadu, India in the month of March. The plant material was taxonomically identified and authenticated by V.Chelladurai (Research Officer) Botany (C.C.R.A.S) Government of India.

Extraction

The aerial part of *Atropa acuminata was* dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol (90% v/v) in soxhlet apparatus at 60°C[16]. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in a vacuum desiccator.

GC–MS analysis of ethanol extract of *Atropa acuminata* for the identification of chemical composition

The identification of chemical composition of ethanol extract of *Atropa acuminata* was performed using a GC–MS spectrograph (Agilent 6890/Hewlett–Packard 5975) fitted with electron impact (EI) mode. The ethanol extract (2.0 mL) of *Atropa acuminata* was injected with a Hamilton syringe to the GC–MS manually for total ion chromato- graphic analysis in split mode. In quantitative analysis, selected ion monitoring (SIM) mode was employed during the GC–MS analysis. SIM plot of the ion current resulting from very small mass range with only compounds of the selected mass were detected and plotted.

Animals

Studies were carried out using Wistar albino male rats (150-200g), obtained from Indian Veterinary Preventive medicine (IVPM), Ranipet, Tamilnadu, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by Poultry Research Station, Nandhanam, India and fresh water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee.

Drugs and Chemicals

Silymarin was purchased from Micro labs, Tamilnadu, India. Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin and total protein kits were procured from Span Diagnostics, Surat, India, and the rest of the chemicals utilized were of analytical grade and were obtained from Ranbaxy research laboratory, Hyderabad, India.

Experimental treatments

Animals were divided into five groups of six animals each. Group I treated with vehicle (distilled water) was kept as normal. Group II treated with a single dose of acetaminophen (APAP) of 750mg/kg body weight was kept as toxin control. Group III and IV were treated with ethanol extract of *Atropa acuminata at* two different doses of 250 and 500 mg/kg body wt plus APAP. Group V were fed with standard drug silymarin 25mg/kg daily for seven days. The extract was administered by oral gavages 1 h before APAP administration [17].

Preparation of serum from blood

After 24 h, animals were sacrificed by chloroform anaesthesia. Blood was collected by heart puncture. The blood samples of each animal were taken and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 600×*g* for 15 min and analyzed for various biochemical parameters including serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT),[18] alkaline phosphatase (ALP) ,[19] bilirubin [20] and total protein [21].

Preparation of liver homogenate

Hepatic tissues were homogenized in KCl [10 mM] phosphate buffer (1.15%) with ethylene-diamine tetra acetic acid (EDTA; pH 7.4) and centrifuged at 12,000×g for 60 min. The supernatant was used for assay of the marker enzymes (glutathione peroxidase, glutathione-s-transferase, superoxide dismutase and catalase), reduced glutathione, thiobarbituric acid reactive substances (TBARS) content, and protein estimation.

Biochemical estimation of markers of oxidative stress

MDA content was Measured according to the earlier method reported [22]. SOD activity was determined according to previous report [23].CAT activity was determined from the rate of decomposition of H_2O_2 by the reported method [24]. GPX activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3 [25]. Glutathione reductase activity was assayed according to previous reports [26, 27]. Protein content in the tissue was determined by earlier method reported [28], using bovine serum albumin (BSA) as the standard.

Histopathological study

On completion of closing regimen animals were sacrificed and the liver dissected out. Paraffin sections were prepared for histological examination and following standard procedure [29]. Hematoxylin-eosin stained sections were observed.

Statistical analysis

The obtained results were analyzed for statistical significance using one way ANOVA followed by Dunnet test using the graph pad statistical software for comparison with control group and acetaminophen treated group. P < 0.05 was considered as significant.

RESULTS

Phytochemical analysis

The ethanol extract of *Atropa acuminata* was a complex mixture of many constituents and 20 compounds were identified in this plant by GC–MS. Phytoconstituents such as Glycerin, Thymine, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 2-Furancarboxaldehyde, ,5-(hydroxymethyl)-, 2-Coumaranone, 1,2,3-Benzenetriol, Sucrose, 1,2,3,4-Cyclohexanetetrol, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Phenol, 2,4,6-trimethyl-,5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol, Dibutyl phthalate, Phytol, Hexanedioic acid, bis(2-ethylhexyl) ester, 1,2-Benzenedicarboxylic acid, diisooctyl ester, Tetrazol-5-amine, N-(3,4-dimethoxybenzyl)-, 4,13,20-Tri-O-methylphorbol 12-acetate, Bufa-20,22-dienolide, 14,15-epoxy-3,16-dihydroxy-, (3á,5á,15á,16á)- [Synonyms: Cinobufagin, deacetyl-],2-Allyl-3,6-dimethoxybenzyl alcohol, 1,2-Dimethoxy-4-n-propylbenzene were identified in the ethanol extract of *Atropa acuminata* by relating to the 185 corresponding peak area through coupled GC–MS [Table 1; Figure 1].

Biochemical analysis

The effect of *Atropa acuminata* on serum marker enzymes is presented in Figure 2, 3 & 5. The serum levels of GOT, GPT, ALP and total bilirubin were markedly significantly ($p< 0.01$) elevated and that of protein levels were significantly ($p< 0.01$) decreased in acetaminophen treated animals, indicating liver damage. Administration of ethanol extract of *Atropa acuminata at* the doses of 250 and 500 mg/kg remarkably significantly ($p< 0.05$; $p< 0.01$) prevented hepatotoxicity induced by acetaminophen.

Acetaminophen treatment caused a significant ($P<0.01$) decrease in the level of SOD, catalase, GPX and GST in liver tissue when compared with control group. The treatment of *Atropa acuminata* at the doses of 250 and 500 mg/kg resulted in a significant ($P<0.05$; $P<0.01$) increase of SOD, catalase, GPX and GST when compared to Group II [Figure 3 & 4]. The standard drug, silymarin treated animals also showed a significant ($P<0.01$) increase in antioxidant enzymes levels compared to Group II.

Analysis of MDA levels by thiobarbituric acid reaction showed a significant ($P<0.01$) increase in the acetaminophen treated rats. Treatment with *Atropa acuminata* (250 mg/kg & 500 mg/kg) significantly ($P<0.01$; $P<0.01$) prevented the increase in MDA level which was brought to near normal [Figure 2]. Acetaminophen treatment caused a significant ($P<0.01$) decrease in the level of SOD, catalase, GPX and GST in liver tissue when compared with control group.

Histopathological studies

Morphological observations showed an increased size and enlargement of the liver in acetaminophen treated groups. These changes were reversed by treatment with silymarin and also *Atropa acuminata* at the two different doses tested groups. Histopathological profile of the normal animal showed normal hepatocytes with well-preserved cytoplasm and there was no sign of inflammation, which has been illustrated in Figure 6 a. The acetaminophen treated animals showed severe centrilobular necrosis and fatty infiltration [Figure 6 b]. Treatment with different doses of ethanol extract of *Atropa acuminata* and silymarin produced mild degenerative changes and absence of centrilobular necrosis when compared with control [Figure 6 c, d, & e]. All these results indicate a hepatoprotective potential by the *Atropa acuminata*.

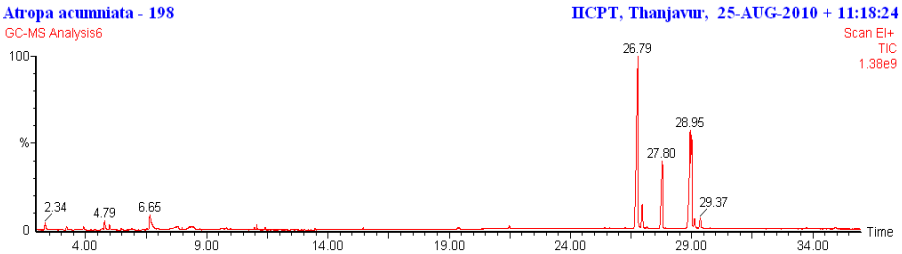


Fig. 1: GC-MS analysis of ethanolic *Atropa acuminata* leaf extract.

The chromatogram showing Tri-O-methylphorbol 12-acetate (26.96), Bufa-20, 22-dienolide, 14, 15-epoxy-3, 16-dihydroxy (27.80) and 2-Allyl-3, 6-dimethoxybenzyl alcohol (29.13) peaks detected by GC-MS

Table 1: Identification of Phyto components in the ethanol extract of *Atropa acuminata* by GC MS analysis

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compounds Nature
1.	2.34	Glycerin	C3H8O3	92	2.29	Alcohol
2.	3.22	Thymine	C5H6N2O2	126	0.97	DNA fraction
3.	3.92	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	144	0.64	Flavonoid fraction
4.	4.79	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	C6H6O3	126	1.85	Aldehyde compound
5.	4.99	2-Coumaranone	C8H6O2	134	0.79	Coumaranone compound
6.	6.65	1,2,3-Benzenetriol	C6H6O3	126	6.76	Pyrogallol
7.	7.81	Sucrose	C12H22O11	342	2.73	Sugar moiety
8.	8.34	1,2,3,4-Cyclohexanetetrol	C6H12O4	148	3.33	Poly Hydroxy benzene
9.	10.98	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C10H12O3	180	0.29	Phenolic compound
10.	11.07	Phenol, 2,4,6-trimethyl-	C9H12O	136	0.48	Phenolic compound
11.	11.41	5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	C11H20O	168	0.25	Alcoholic compound
12.	13.47	Dibutyl phthalate	C16H22O4	278	0.14	Plasticizer compound
13.	15.45	Phytol	C20H40O	296	0.28	Diterpene
14.	19.41	Hexanedioic acid, bis(2-ethylhexyl) ester	C22H42O4	370	0.40	Ester compound
15.	21.48	1,2-Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	390	0.67	Plasticizer compound
16.	26.79	Tetrazol-5-amine, N-(3,4-dimethoxybenzyl)-	C10H13N5O2	235	51.43	Amino compound
17.	26.96	4,13,20-Tri-O-methylphorbol 12-acetate	C25H36O7	448	4.72	Acetate compound
18.	27.80	Bufa-20,22-dienolide, 14,15-epoxy-3,16-dihydroxy-, (3á,5á,15á,16á)- [Synonyms: Cinobufagin, deacetyl-]	C24H32O5	400	17.67	Hydroxy compound
19.	29.13	2-Allyl-3,6-dimethoxybenzyl alcohol	C12H16O3	208	1.71	Alcoholic compound
20.	29.37	1,2-Dimethoxy-4-n-propylbenzene	C11H16O2	180	2.57	Aromatic compound

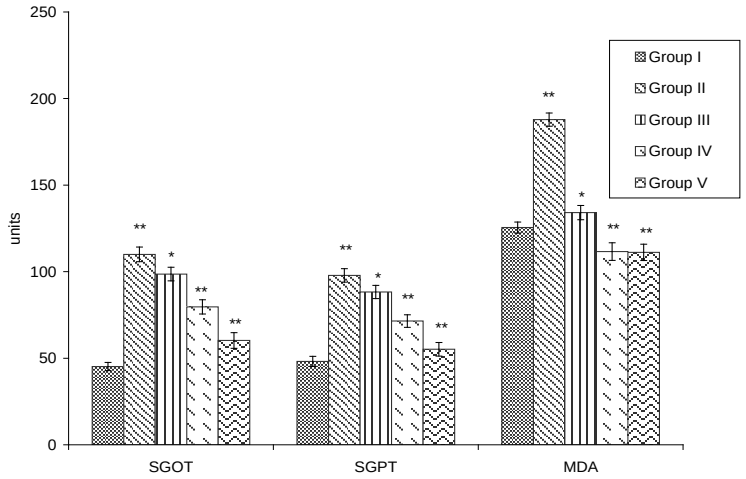


Fig. 2: Effect of ethanolic extract of *Atropa acuminata* and silymarin (standard drug, (25 mg/kg)) on serum levels of SGOT (IU/L), SGPT (IU/L) and MDA (nM/mg of protein) [Lipid peroxidation (LPO)] level of hepatic tissue during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively

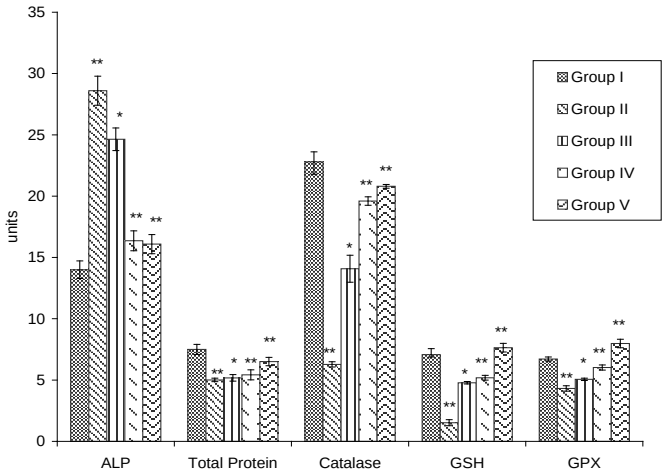


Fig. 3: Effect of ethanolic extract of *Atropa acuminata* and silymarin (standard drug, (25 mg/kg)) on serum levels of alkaline phosphotase (ALP) (IU/L) & total protein and hepatic levels of CAT (U/mg protein), GSH (U/mg protein) and GPX (mg of glutathione utilized/min/mg protein) during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively

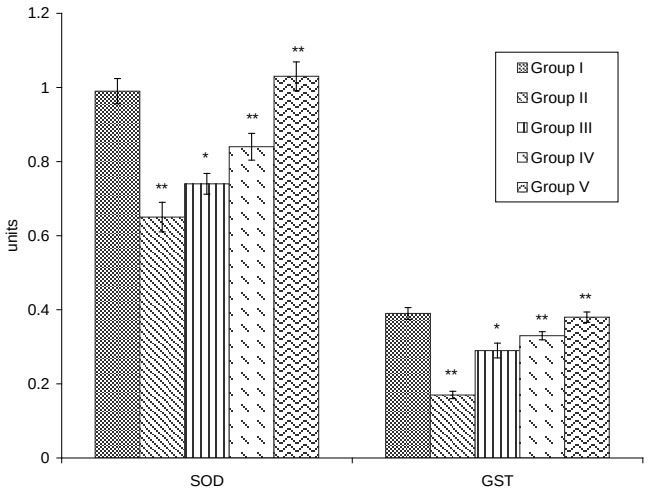


Fig. 4: Effect of ethanolic extract of *Atropa acuminata* and silymarin (standard drug, (25 mg/kg) on hepatic levels of SOD (units of activity/mg protein) & GST (Units/mg protein) during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively

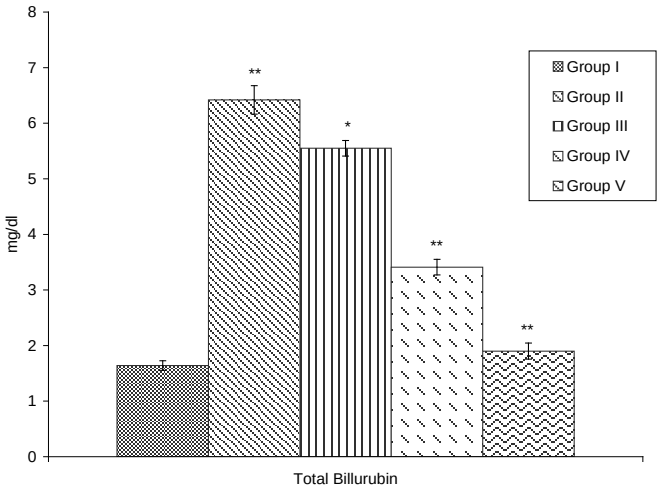
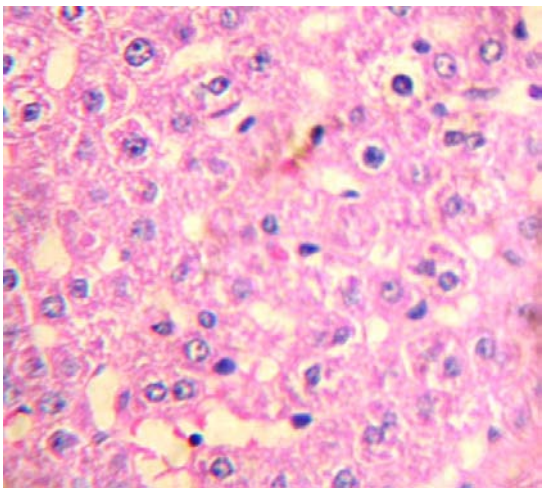
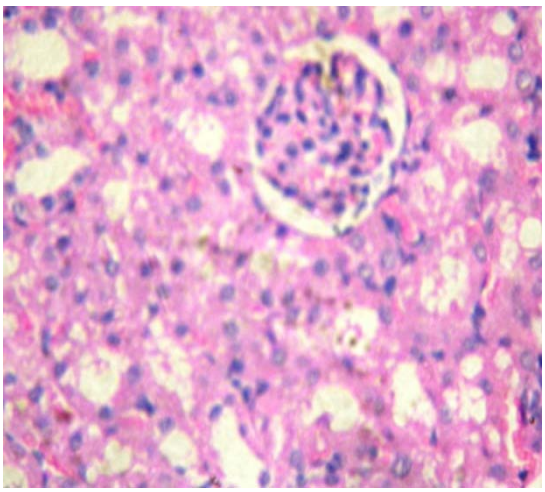


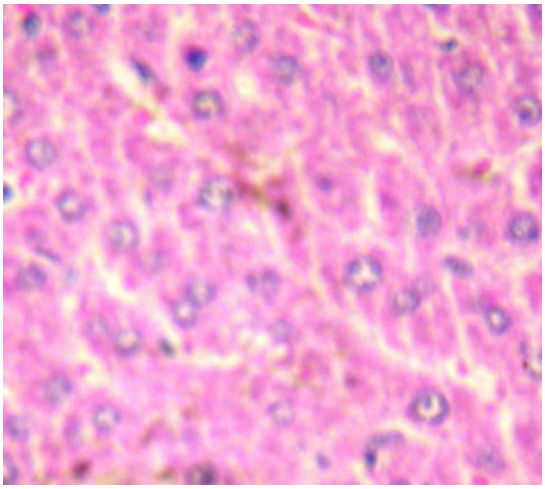
Fig. 5: Effect of ethanolic extract of *Atropa acuminata* and silymarin (standard drug, (25 mg/kg)) on serum levels of total bilirubin (mg/dl) during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively



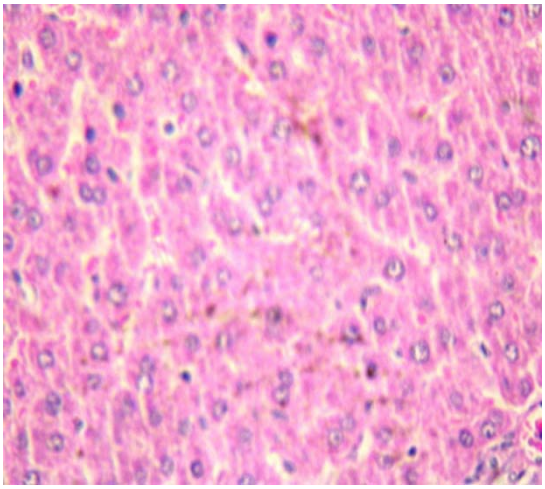
6 (a) Normal photomicrograph of liver tissue of control rat showing normal hepatic cells with central vein and sinusoidal dilation. (H and E 100X)



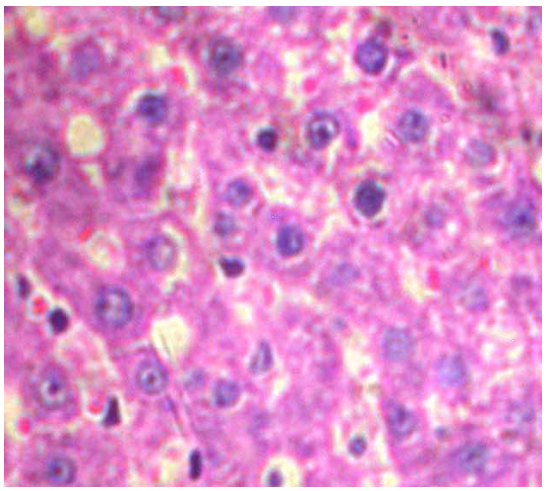
6(b) Liver section of rat showing disarrangement and degeneration of normal hepatic cells with centrilobular necrosis extending to mid zone and sinusoidal hemorrhages and dilation. (H and E 100X)



6(c) Histology of liver from rat which received *Atropa acuminata* ethanolic extract at 250 mg/kg.(Group III) showing mild degenerative changes and absence of centrilobular necrosis. (H and E 100X)



6 (d) Histology of liver from rat which received *Atropa acuminata* ethanolic extract at 500mg/kg(Group IV) showing normal hepatocytes with mild inflammation. (H and E 100X)



6(e) Liver section of Rat treated with silymarin at 25 mg/kg showed less vacuole formation reduced sinusoidal dilation, less disarrangements and degeneration of hepatocytes. (H and E 100X)

DISCUSSION

Acetaminophen (*N*-acetyl-*p*-aminophenol, Paracetamol), a widely used analgesic, antipyretic drug is known to cause hepatotoxicity in experimental animals and humans at high doses [30, 31, 32, 33, 34]. The laboratory features of hepatotoxicity induced by APAP resemble other kinds of acute inflammatory liver disease with prominent increase of GOT, GPT, and ALP levels [35].

In the present study, the serum level of hepatic enzymes GOT, GPT, ALP and total bilirubin levels were increased and reflected the hepatocellular damage in the APAP-induced hepatotoxicity animal model. This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver [36].

However the total protein level was decreased. There was a significant ($P<0.01$) restoration of these enzyme levels on administration of the ethanol extract of *Atropa acuminata* in a dose dependent manner and also by silymarin at a dose of 25 mg/kg. The reversal of increased serum enzymes in acetaminophen induced liver damage by the ethanol extract of *Atropa acuminata* may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [37, 38].

Effective control of ALP, bilirubin and total protein levels points towards an early improvement in the secretary mechanism of the hepatic cells, as well as repair of hepatic tissue damage caused by APAP.

This indicates the anti-lipid per oxidation and/or adaptive nature of the systems as brought about by ethanol extract of *Atropa acuminata* against the damaging effects of free radical produced by APAP.

Previous studies have demonstrated that oxidative stress is a major mechanism in the development of APAP-induced hepatotoxicity [39, 40, 41]. In the present study, the data suggested that high dosage of APAP in the liver could lead to decreased levels of antioxidant enzymes (SOD, CAT, and GPx) and present a significant level of hepatotoxicity in the course of the treatment. However, the *Atropa acuminata* could raise the levels of SOD, CAT, and GPX against the APAP-induced oxidative stress mediated by ROSandRNS.

In APAP overdose, the compound is converted to toxic form, NAPQI (N acetyl-p-benzoquioneimine) which rapidly reacts with glutathione (GSH) and leads to 90% total hepatic GSH depletion[42].

Both reductions of GST & GSH activity APAP-treated rats as observed in this study indicate the damage to the hepatic cells.

Administration of *Atropa acuminata* extract promoted the reactivation of hepatic glutathione reductase enzyme in APAP-treated rats. The restoration of GSH level to such APAP treated rats indicates the protective effect after the administration of ethanol extract of *Atropa acuminata*.

Furthermore, the level of MDA was increased in the group receiving APAP administration, but treatment with the *Atropa acuminata* reduced the amount of MDA. This result indicated that decreasing the formation of lipid peroxidation is also one of the events in preventing the oxidative toxicity by APAP.

In conclusion, ethanol extract of *Atropa acuminata* significantly protects against liver injuries as well as oxidative stress, resulting in improved serum biochemical parameters such as SGOT, SGPT and ALP. The reduced levels of parameters of SOD, CAT, GSH, GPX, and GST in acetaminophen-treated rats were significantly increased by treatment with *Atropa acuminata*. It may be due to synergistic effect of the chemical compounds present in them, making them good source for hepatoprotective and antioxidant activities against APAP induced hepatotoxicity and has the potential for use as a hepatoprotective agent. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

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