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HEPATOPROTECTIVE ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *OPERCULINA TURPETHUM* LINN. AGAINST D-GALACTOSAMINE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The genus Operculina has potential activity as a hepatoprotective agent. The present pharmacological investigation focuses on evaluation of the efficacy of hydroalcoholic extract of roots of *Operculina turpethum* Linn. for their protection against D-Galactosamine (D-GaIN) induced hepatotoxicity. The hydroalcoholic extract of roots of *O.turpethum* Linn. was prepared and phytochemical screening was done. The biochemical investigation *viz.* Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline phosphatase (ALP), Triglycerides (TGL), Total Cholesterol, Albumin, Total Protein and total Bilirubin (TB) was done against D-GaIN induced hepatotoxicity in wistar albino rats. The histopathological studies of liver were also done. The phytochemical screening of the hydroalcoholic extract showed the presence of alkaloids, carbohydrates, glycosides, flavanoids, saponins, tannins and phenolic compounds. Pretreatment with the hydroalcoholic extract of roots significantly prevented the physical, biochemical, histological, and functional changes induced by D-GaIN in the liver. The extract showed significant hepatoprotective effect as evidenced by decreased biochemical parameters like AST, ALT, ALP, TGL Total Cholesterol and TB and elevated levels of Total protein and Albumin which was supported by histopathological studies of liver. The antioxidant parameters and phenobarbitone sleeping time also supported hepatoprotectivity. The hydroalcoholic extract showed significant hepatoprotective activity comparable with standard drug silymarin as well as hepatotoxin D-GaIN. From these results, it is concluded that the *O.turpethum* has potential effectiveness in treating liver damage induced by D-GalN.

Key words: *Operculina turpethum* Linn., Hydroalcoholic extract, Hepatoprotective activity, D-Galactosamine.

INTRODUCTION

Liver plays a vital role in regulation of physiological processes. It has involved in several vital functions, such as bile secretion, metabolism and storage. The detoxification of a variety of drugs and xenoboitics occur in the liver. Liver disorders may be classified as acute (or) chronic hepatitis (inflammatory liver disease), hepatosis (non inflammatory diseases) and cirrhosis (Degenerative disorder resulting in fibrosis of liver) (Subramoniam A and Pushpangadam P, 1999).

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Due to liver disorders every year 20,000 deaths occur. Viruses, excessive drug therapy and alcohol intoxication are main causes of liver diseases (Chattopadhay RR *et al*., 1992; Bhattacharya SK *et al*., 2007). The rate of hepatotoxicity has been reported to be much higher in developing countries like India (8 to 30%) compared to that in advanced countries (2%-3%) (Sharma SK, 2004). According to the survey of Hepatotoxicity Clinical Research Network, U.S.A as recorded 100000 deaths for year and are mainly due to abounded usage of drugs. Further, hepatotoxicity is ranking as the most frequent with acute liver failure (Karthi Kumar S *et al*., 2009).

Liver diseases are mainly caused by toxic chemicals, aflatoxin, certain antibiotics, peroxidised oil, chemotherapeutics, carbon tetrachloride, acetaminophen,

pesticides etc, environmental toxins, excess consumption of alcohol, viral infections and auto immune disorders (Pandey Govind P, 1980; Pandey Govind P, 1990; Kumar CH *et al*., 2011). Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. The increased lipid peroxidation produced during the liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis. About 90% of the acute hepatitis is due to viruses. The major viral agents involved are hepatitis A, B, C, D (delta agents) E and G. Hepatitis B infection often results in chronic liver diseases and cirrhosis of liver. Primarily liver cancer has also been shown to be produced by these viruses (Recknagel RO *et al*., 1983). Drugs are an important cause on liver injury. Drug induced liver injury is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies (Michael P, 2006).

In spite of the tremendous advances made in conventional drugs, no effective hepatoprotective drug is available. Herbal drugs are known to play an important role in the treatment of liver disorders. There are numerous herbals and polyherbal formulations claimed to have hepatoprotective activity. Nearly 160 Phyto-constituents from 101 plants have been claimed to possess liver protecting activity from ancient times (Doreswamy R and Sharma D, 1995).

MATERIALS AND METHODS

Collection and authentication

Operculina turpethum roots were procured from Srinivasa ayurvedic pharmacy, TTD, Narsingapuram, Tirupati. The plants were authenticated by Dr.K.Madhava Chetty M.SC., M.Phil., Ph.D, Associate Professor, Dept. of Botany, Sri Venkateswara University, Tirupati, A.P, India.

Processing of the plant materials

The material was washed with water, cut into pieces, dried under shade for about two weeks. The dried plant material was then pulverized into coarse powder in a grinding machine. The powder obtained was passed through No: 40 mesh and used for extraction and to determine the quality control profiles.

Preparation of extracts

The dried plant material was extracted separately with hydroalcoholic solvent (ethanol 70 parts:water 30 parts).Solvent from sample was filtered, squeezed off and evaporated off under reduced pressure in a rotary evaporator to obtain crude extracts and kept under refrigeration.

Preliminary phytochemical screening

The chemical tests were carried out to identify the various phytoconstituents present in the hydroalcoholic extract of roots of *O.turpethum* Linn. Hydroalcoholic extract of roots of *O.turpethum* Linn. was subjected to qualitative chemical tests for the detection of various phytoconstituents such as alkaloids, carbohydrates, glycosides, flavanoids, saponins, tannins and phenolic compounds.

Selection and maintenance of animals

All the protocols used in this study were approved by Institutional Animal Ethical Committee (IAEC) of Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati, A.P, India (Registered No.1016/a/06/CPCSEA-003/2008). Healthy adult male albino rats of Wistar strain weighing 150-220 g were procured from M/s Raghavendra enterprises, Bangalore. The animals were acclimatised for 7 days under standard housing conditions (24 ºC ± 1 ºC; 45-55% RH with 12:12 h light/dark cycle).Animals had free access to rat pellet feed supplied by M/s. Hindustan Lever Ltd., Bangalore, India and water *ad libitum*. The animals were placed separately in large spacious hygienic polypropylene cages during the course of the experimental period.

Acute toxicity studies (Ecobiochon DJ, 1997)

The acute toxicity study was aimed at establishing the therapeutic index i.e. the ratio between the pharmacologically effective dose, lethal dose and also to perform the primary screening.

Toxicity study was carried as per internationally accepted protocol drawn under OECD 423 guidelines in wistar rats at five dose levels of 5, 50, 300, 2000 and 4000 mg/kg b.wt. Acute toxicity studies of the extracts of plants were carried as follows.

Wistar Rats were fasted for over night and maintained with water *ad libitum.* The rats were separated into five groups of six in each. The extracts were administered upto a dose level of 4000 mg/kg per rat orally as a fine suspension in 1% CMC.

After administration of the test compounds, animals were observed individually and continuously for 30 mins, 2 and 24 h to detect changes in the autonomic or behavioural responses and also for tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and then monitored for any mortality for the following 14 days. A group of animals treated with vehicle served as control.

Induction of hepatotoxicity

D-Galactosamine induction

The liver damage (hepatotoxicity) in Wistar albino rats was induced by a single i.p administration of D(+) galactosamine (400 mg/kg b.w.) in sterile water for injection on 14th day of experiment (Deshpande UR *et al*., 2003; Fatma M Lebda *et al*., 2011).

Standard

Silymarin

Silymarin, a known hepatoprotective was used as reference standard for comparison at a dose of 50 mg/kg b.w in 1% CMC orally, daily for 14 days (Detlef Schuppan *et al*., 1999).

Experimental design

The experimental design of the investigation was carried out in the following regimen. The animals were divided into five groups. Each group consist of six animals (n=6). Except normal group, all the other groups were given hepatotoxicant (D-GalN on 14^{th} day i.p). The plant extract and standard drug are administered daily, orally for 14 days. All the parameters of the present investigation were measured on 15^{th} day (Table 1).

Estimation of biochemical parameters

Blood and liver samples were collected after the experimental period of 14 days.All the rats were aneasthetized with pentathol sodium. For the estimation of various biochemical parameters blood samples were collected by sino-orbital puncture in Eppendroff's tubes (1 ml) containing anti-coagulant (11% sodium citrate solution). Plasma was separated by centrifuging at 6000 rpm for 15 mins and estimated in Autoanalyzer Microlab 200 by using kits (Ecoline) supplied by MERCK Ltd., Mumbai. The rats were sacrificed after the collection of blood samples and the livers were excised immediately for histopathological examination.

- **Biochemical studies**
  Physical parameter weight of the liver
- **Biochemical parameters**
  (a) Estimation of aspartate amino transaminase (AST)
  (b) Estimation of alanine amino transaminase (ALT)
  (c) Estimation of alkaline phosphatase (ALP)
  (d) Estimation of total bilirubin (TB)
  (e) Estimation of total proteins (TP)
  (f) Estimation of total cholesterol (TC)
  (g) Estimation of triglycerides (TGL)
- **Tissue anti-oxidants and pro-oxidants**
  (a) Superoxide dismutase (SOD)
  (b) Catalase (CAT)
  (c) Glutathione reductase (GSH)
  (d) Malondialdehyde (MDA)
- **Functional parameters**
  - Phenobarbitone induced sleeping time
- **Histopathology**
  - Liver

Histopathological studies of liver

The livers were excised quickly and kept in 10% buffered neutral formalin. The material was processed by standard methods. Paraffin blocks were made and sections were cut. These sections were stained with heamotoxylin and eosin and mounted on the glass slides. The histopathological changes were observed and recorded.

The liver tissues were fixed in 10% formalin (i.e 4% formaldehyde) for 1 h. The tissues were subsequently processed which include 3 changes in absolute isopropyl alcohol (for dehydration), 3 changes in xylene (for cleaning) and 2 changes in molten paraffin wax. Subsequently paraffin blocks of the tissue were made, thin sections of 5 μ thickness were cut with a rotary microtome, sections taken onto the slides, deparaffinized, cleared, rehydrated and finally stained with haematoxylin (Harris) and Eosin. The stained sections were mounted. After appropriate labeling the slides were interpreted by the pathologist.

RESULTS

Acute toxicity studies

In the acute toxicity studies, it was observed that none of the doses (i.e 5, 50, 300, 2000 and 4000 mg/kg b.wt) produced any lethality among the tested animals as a single dose and considered safe and dose was fixed at $1/10^{th}$ of maximum test dose (200 mg and 400mg/kg b.wt).

Physical parameter

There was a significant decrease in liver weight in D-Galactosamine insult group when compared to the normal group. The hydro alcoholic plant extract and Silymarin of present study restored liver weights to near normal liver weight (Table 2).

Biochemical analysis

Measurement of serum biomarkers represent an index for the assessment of hepatic function and in present study a significant increase in AST, ALT, ALP, TGL, TC and TB levels in the D-Galactosamine insult group when compared to the normal group. The hydro alcoholic plant extract and Silymarin showed a significant reduction in the AST, ALT, ALP, TGL, TC and TB levels when compared to D-Galactosamine groups. There was a significant decrease in the TP, albumin levels in the D-Galactosamine group when compared to the normal group. Test and Silymarin groups showed a significant increase in TP and albumin levels when compared to D-Galactosamine group (Table 3 and 4).

Tissue anti-oxidants and pro-oxidants

There was a significant fall in SOD, Catalase, GSH levels and significant increase in lipid peroxidation in the D-galactosamine group when compared to the normal group. Test and Silymarin groups showed a significant rise in the SOD, Catalase and GSH levels when compared to D-galactosamine group and significant

Table 1. Experimental design of D-GalN induced hepatotoxicity

Group	Treatment	*Operculina turpethum* Linn.
1	Normal control	1% CMC 1 ml/kg p.o
2	D-GalN control	400 mg/kg i.p (on 14th Day)
3	D-GalN + Silymarin control	400 mg/kg i.p +50 mg/kg p.o (Daily)
4	Test I	D-GalN 400 mg/kg i.p + 200mg/kg p.o (Daily)
5	Test II	D-GalN 400mg/kg i.p + 400 mg/kg p.o (Daily)

Table 2. Effect of hydroalcoholic extract of *O. turpethum* on liver weight

D-GalN 400 mg/kg i.p	
Vehicle control (1 ml/kg)	2.41±0.34
D-GalN control (400 mg/kg)	1.95±0.06
D-GalN + Sylimarin control (50 mg/kg)	2.36±0.05
D-GalN + *O. turpethum* (200 mg/kg)	2.0±0.21
D-GalN + *O. turpethum* (400 mg/kg)	2.11±0.06

Table 3. Effect of hydroalcoholic extract of *O. turpethum* biochemical parameters in D-GalN induced hepatotoxic rats

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	TGL (mg/dl)
Vehicle control (1 ml/kg)	28.33±1.48	18.83±0.60	197.67±6.82	79.83±0.87
D-GalN control (400 mg/kg)	73.33±3.40**	84.83±3.44**	373.33±17.7**	125.67±1.76**
D-GalN + Silymarin control (50 mg/kg)	31.83±2.02***	23.17±0.95***	215.17±6.46***	71.17±2.27***
D-GalN + Test1 (200mg/kg) *O. turpethum*	64.65±8.434	77.50±2.75	302.00±19.94	89.17±1.74***
D-GalN + Test2 (400mg/kg) *O. turpethum*	56.33±1.80***	68.67±2.28***	273.0±12.58***	83.71±3.11***

All Values shown are mean ± SEM and n=6
Superscript** denotes statistical significance in comparison to solvent control group at $p < 0.01$
Superscript *** denotes statistical significance in comparison to hepatotoxicant group at $p < 0.01$

Table 4. Effect of hydroalcoholic extract of *O. turpethum* biochemical parameters in D-GalN induced hepatotoxic rats

Groups	Total cholesterol (mg/dl)	Albumin (g/dl)	Total protein (g/dl)	Total bilirubin (mg/dl)
Vehicle control (1 ml/kg)	48.00±1.21	5.18±0.11	6.90±0.15	0.48±0.10
D-GalN control (400 mg/kg)	174.0±2.96**	3.55±0.16**	4.35±0.40**	1.03±0.07**
D-GalN + Silymarin (50 mg/kg)	69.432±7.543***	4.42±0.14$^{\#}$	6.0±0.12***	0.42±0.09***
D-GalN + Test1 (200mg/kg) *O. turpethum*	114.67±3.81$^{\#}$	3.79±0.11	5.07±0.50	0.43±0.07***
D-GalN + Test2 (400mg/kg) *O. turpethum*	91.83±1.50$^{\#}$	3.83±0.36	5.87±0.23$^{\#}$	0.40±0.07***

All Values shown are mean ± SEM and n=6
Superscript** denotes statistical significance in comparison to solvent control group at $p < 0.01$
Superscript *** denotes statistical significance in comparison to hepatotoxicant group at $p < 0.01$
Superscript $^{\#}$ denotes statistical significance in comparison to hepatotoxicant group at $p < 0.05$

Table 5. Effect of hydroalcoholic extract of *O. turpethum* on tissue parameters in D-GalN induced hepatotoxic rats

Groups	SOD (U/mg protein)	CAT (µM H_2O_2 consumed/mg protein)	Reduced GSH (µg of GSH / mg protein)	MDA (nM of MDA/mg protein)
Vehicle control (1 ml/kg)	5.34±0.05	7.37±0.43	8.64±0.32	0.71±0.07
D-GalN control (400 mg/kg)	1.32±0.58**	1.03±0.03**	2.34±0.31**	2.89±0.02**
D-GalN + Silymarin (50 mg/kg)	4.76±0.54***	5.53±0.53**	6.21±0.59***	0.82±0.07***
D-GalN + Test1 (200mg/kg) *O. turpethum*	2.01±0.23	2.67±0.90$^{\#}$	2.92±0.64	2.01±0.28
D-GalN + Test2 (400mg/kg) *O. turpethum*	2.99±0.49	5.39±0.60***	4.02±0.19***	0.81±0.09***

All Values shown are mean ± SEM and n=6
Superscript** denotes statistical significance in comparison to solvent control group at $p < 0.01$
Superscript *** denotes statistical significance in comparison to hepatotoxicant group at $p < 0.01$

Table 6. Effect of hydroalcoholic extract of *O. turpethum* on phenobarbitone induced sleeping time in D-GalN induced hepatotoxic rats (min)

Groups	*Operculina turpethum*
Vehicle control (1 ml/kg)	134±4.69
D-GalN control(400 mg/kg)	203±9.91**
Silymarin control(50 mg/kg)	120±9.08***
Test-I (200 mg/kg)	152±8.42***

All values are shown as mean ± SEM and n=6.
Superscript ** denotes statistical significance in comparison to solvent control group at $p < 0.01$
Superscript *** denotes statistical significance in comparison to hepatotoxicant group at $p < 0.01$

Histopathological observations of rat liver samples (D- GalN)

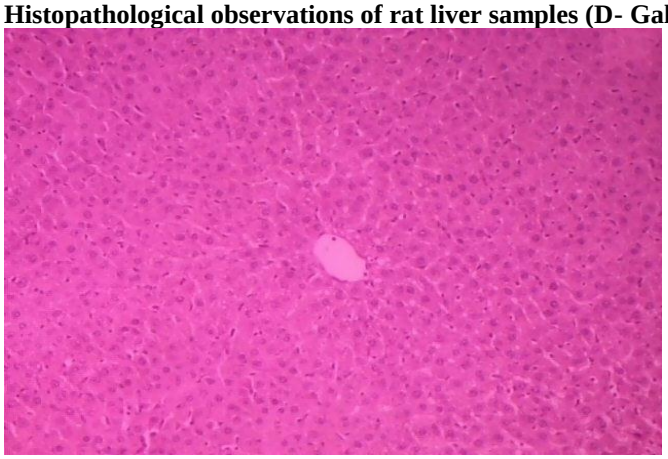
Fig. 1

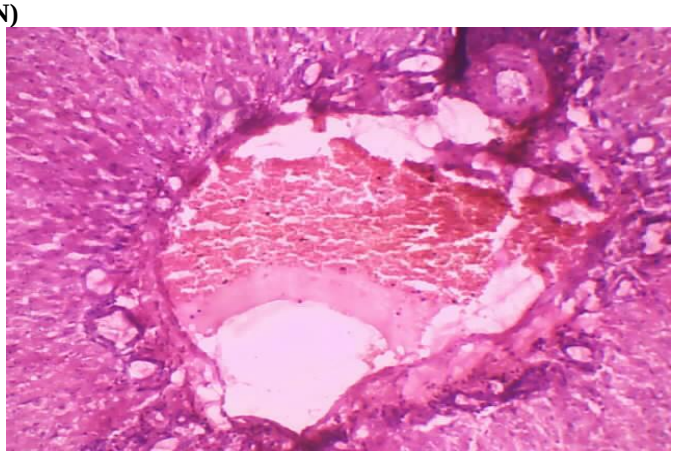
Fig. 2

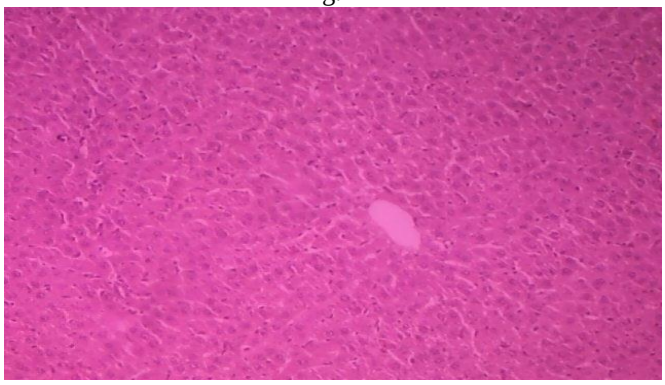
Fig. 3

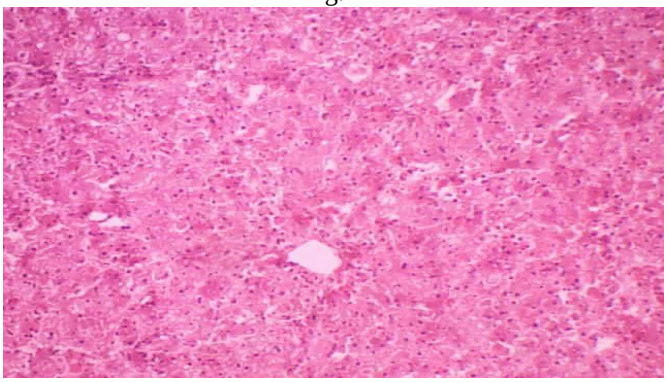
Fig. 4

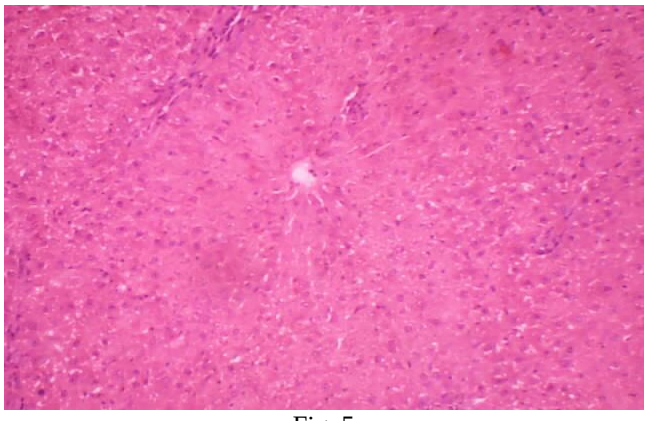
Fig. 5

decrease in the lipid peroxidation implicating protection against hepatocellular damage by hydroalcoholic extract and Silymarin (Table 5).

Functional parameters

Estimation of Phenobarbitone induced sleeping time in rats reveals liver metabolic function. Administration of Phenobarbitone in D-Galactosamine group increased sleeping time due to alteration of liver metabolic function and there was a significant reduction in sleeping time in test and Silymarin groups (Table 6).

Histopathological studies

Histopathology findings of liver (D-Galactosamine)

Control rats treated with CMC (Fig. 1)

Sections show structure of liver whose architecture is well preserved. The hepatocytes are normal. The central vein appear normal and the hepatocytes appear to be arranged radiating away from it. The portal tracts composed of portal vein, radical hepatic arteriole and bile duct radical are seen. However no liver cell injury /necrosis are seen.

Rats treated with D-Galactosamine (Fig. 2)

Sections show liver tissue which displays mild lobular disarray with inflammatory cells in the sinusoids of the lobules. The hepatocytes show ballooning degeneration as well as focal necrosis within lobules. Randomly scattered councilman bodies suggestive of apoptosis are seen. Most of the portal tracts show intense periportal inflammation. Some of the portal tracts show bile duct hyperplasia.

Rats treated with D-GalN and Silymarin (Fig. 3)

Sections show findings of thickening of hepatocyte cords in some areas. Silymarin has produced only minimal reversal of effects of D-GalN-bit, there is regenerative activity is addition, which may eventually lead to restoration of normal architecture and function.

Rats treated with D-GaIN and hydroalcoholic extract of *Operculina turpethum* in doses of 200 mg and 400 mg, respectively (Fig. 4 and 5)

Sections studied show near to normal architecture of liver. Both the doses showed similar effects such as absence of necrosis, council man bodies and balloon degeneration. Portal triaditis and sinusoidal dilatation with congestion was seen in both groups.

DISCUSSION

D-GalN-induced hepatic damage is a useful model for the study on hepatic injury. It has been shown to produce liver damage similar to human viral hepatitis in morphological features (Decker K *et al*., 1974). D-GalN induces a rapid decrease in uracil nucleotides which ultimately causes a rapid inhibition of RNA synthesis, disturbance of biosynthesis of glycoproteins, lipoproteins and nucleic acids with alteration of cellular membrane and leakage of enzymes into serum, leads to progressive damage of cellular membrane and ultimately to spotty liver-cell necrosis. This cellular damage provokes inflammatory reactions resulting in a histological picture closely resembling human viral hepatitis (Decker K *et al*., 1971). The changes in the structure of cellular membrane may induce lipid peroxidation due to the liberation of active free radicals.

In the present study, it was observed that D-GalN hepatotoxicity decreases the weight of liver due to the inhibition of protein synthesis, necrosis and leakage of enzymes from the liver. The extract of *Operculina turpethum* restored the liver weight by promoting the regeneration of hepatocytes (Girish C *et al*., 2009).

Liver damage leads to high serum levels of AST and ALT which are released due to the leakage of cell membrane from liver into the blood. Among the two enzymes, AST is a better index of liver injury, as liver ALT activity represents 90 % of total enzyme present in the body (Achiliya GS *et al*., 2003). ALP is a membrane bound glycoprotein produced in bile duct and is another parameter of liver damage. Increase in its activity due to increased synthesis in presence of increased biliary pressure (Suresh Kumar SV *et al*., 2006). Reduction in the levels of AST and ALT towards near to normal values is an indicator of stabilization of plasma membranes as well as normalization of liver cell damage caused by D-galN injury. This effect is in line with serum levels of the AST and ALT attenuate to near normal with healing of the hepatic parenchyma and regeneration of hepatocytes. Suppression of elevated serum activity with reduction of abnormal bilirubin levels suggests biliary dysfunction during hepatic injury due to D-galN (Mukherjee PK, 2002).

The present study showed that D-GalN caused significant decreased levels of total proteins and serum albumin indicating hepatic disorder (Wong MC *et al*., 2007). It was reported that the lowering of serum albumin levels may contribute to reduction of albumin mRNA expression (Dabeva MD *et al*., 1993). The elevated levels of total proteins & albumin by the extract of the *Operculina turpethum* Linn., in pretreated and standard groups increased due to protein synthesis (Girish C *et al*., 2009), stability of the hepatic cell membrane structure and stimulation of albumin mRNA expression indicates hepatoprotective activity compared to control rats and restoring liver vitality.

The activity of antioxidant enzymes SOD and CAT has significantly reduced in D-GalN treated group (Anandan R *et al*., 1999; Meena B *et al*., 2008) compared to the normal group. Both enzymes are restored to near normal level in pretreated animals due to the scavenging

of free radicals produced by D-GalN induced lipid peroxidation.

In this study, it was observed that GSH was depleted in D-GalN treated rats and is correlated with report and may be due to more consumption of GSH by increased free radicals. The depletion of GSH is suppressed in the pretreatment of rats by *Operculina turpethum* extract and standard compared to hepatotoxicant group.

The levels of MDA in lipid peroxidation significantly increased in animals intoxicated with D-GalN (Wong MC *et al*., 2007). Elevation of MDA levels also shows parallel significant reduction in GSH. The decreased level of MDA was observed by the plant extract treated animals.

Barbiturates are exclusively metabolized by the hepatic microsomal enzymes. D-GalN induced hepatic injury decreased the activity of hepatic microsomal enzymes that delay barbiturate metabolism thereby prolonging the sleeping time which is restored by the plant extract.

These findings could be correlated with an earlier study where it was reported that pretreatment of rats with *Tephrosia purpurea* offered heptoprotective action against D-galactosamine induced toxicity (Sree Ramamurthy M *et al*., 1993) and hepatoprotective effect of *Sargassum polycystum* was evaluated in D-galactosamine induced hepatitis. It significantly reduced the diagnostic marker enzymes (AST, ALT and ALP) in plasma of rats. It has also demonstrated the antioxidant activity against D-GalN induced hepatitis by inhibiting the activation of lipid peroxidation and by preserving the enzymatic and non-enzymatic antioxidant defense system at near normal. The hepatoprotectivity might be due to its antioxidant property and membrane stabilizing action (Meena B *et al*., 2008).

CONCLUSION

As a conclusion, the results indicate that the hydroalcoholic extract of *O.turpethum*, had significant hepatoprotective activity and diminished the extent of liver injury induced by D-GalN and contributed to improvement of condition for restoration of liver hepatocytes and its function following regeneration process. The different bioactive constituents in the extract may have different mechanism of actions for curing the liver disorders and in the combined form may exhibit additive or enhanced activity.

There is a need to isolate and purify the active principles involved in these plants and to confirm the mechanism for providing scientific basis for its usage in the traditional system of medicine and management of liver disorders.

The present study thus provided more insight in to the mechanism of hepatoprotective action of *O. turpethum* and also provides scientific basis for its usage in the traditional system of medicine and for the management of hepatotoxicity.

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