Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article
Anticholesterolemic, Hepatoprotective and Antioxidant activity of *Glinus lotoides* linn. against ethanol induced liver damage in rats

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ABSTRACT - The aim of the present study was to investigate the effect of ethanolic extract of *Glinus lotoides* Linn. (EEGL) against ethanol induced lipid peroxidation. Administration of ethanol (18%) for 45 days induces lipid peroxidation leading to liver damage. Hence the enzyme levels such AST, which are good markers of liver condition have been elevated and also the triglycerides and cholesterol. Simultaneous administration of EEGL at the dose of 200 mg/kg and 400 mg/kg reduced these elevated parameters significantly ($p<0.001$) substantiating that EEGL is inhibiting the ethanol induced liver damage in rats. This was further supported by the evidence of significantly increased ($p<0.05$, $p<0.01$ respectively) glutathione level indicating increased antioxidant enzyme level and significantly ($p<0.001$) decreased malondialdehyde level indicating the decreased oxidative stress.
KEYWORDS - *Glinus lotoides*; Hepatoprotective; Lipid peroxidation; Antioxidants.

INTRODUCTION

Glinus lotodies Linn. is a plant belonging to the family Aizoceae and widely distributed throughout India (1). This plant is reported to possess various medicinal uses. It is used as antiseptic, anthelmentic, against diarrhoea, bilious attack, for curing boils, wound and relieving pain (2). The juice of this plant is taken internally to strengthen weak children (3). It also has an anti-tumour activity (4). Chronic intake of alcohol is found to produce hypercholesterolaemia and hypertriglyceridemia (5) and this induces free radical generation which is a common cause for liver damage. In the present study an attempt was made to establish the effect of EEGL against ethanol induced liver damage and lipid peroxidation.

MATERIALS AND METHODS

Preparation of the Extract

The whole plant parts (1 kg) of *Glinus lotodies* Linn. were shade dried, pulverized and extracted with alcohol (1:10) for 72 hours. The yield was 50 g. The extract was phytochemically investigated (6).

Animals

The study was carried out after obtaining clearance from the Institutional Animal Ethics Committee (IAEC) and CPCSEA guidelines were adhered to throughout the study.

Albino Wistar rats (either sex) weighing 125-150 g were acclimatized to our laboratory conditions. They were housed in plastic cages, maintained under 12 h dark light cycle, fed with standard pellet diet and water *ad libitum*.

Acute toxicity studies

Acute toxicity studies were carried out according to OECD guidelines (7) and the extract was found to be safe up to 2000 mg/kg.

Hepatoprotective activity

The animals were divided into four groups viz. G1, G2, G3 and G4. Lipid peroxidation was induced in G2, G3 and G4 following the method of Mahendran *et al* (8), whereas G1 served as normal control.

Treatment Protocol

- Group 1 (G1) – Normal control.
- Group 2 (G2) – Rats treated with 5 ml/100 g of 18% ethanol for 45 days.
- Group 3 (G3) – Rats treated with 5 ml/100 g of 18% ethanol and 200 mg/kg of EEGL for 45 days.
- Group 4 (G4) – Rats treated with 5ml/100 g of 18% ethanol and 400 mg/kg of EEGL for 45 days.

On day 45, the overnight fasted rats were anesthetized and blood was collected by retroorbital puncture. Serum was analysed for triglycerides and total cholesterol to evaluate its effect on hyperlipidemia.

Serum level of enzymes AST and ALT were also estimated to study its effect on liver damage.

Estimation of peroxidation product and antioxidant enzyme

The level of peroxidation product viz. Malondialdehyde (MDA) was measured in blood (9) where the reaction depends on the formation of a coloured complex between malondialdehyde (MDA) and thiobarbituric acid (TBA) having an absorption maximum at 532 nm.

Similarly the level of glutathione (GSH) content in blood was measured (10) where 5-5[1]-Dithiobis 2-nitro benzoic acid (DTNB) is reduced by glutathione, forming highly coloured yellow anion. The optical density of this yellow substance is measured at 412 nm.

Statistical analysis

Results were expressed as mean ± SEM and evaluated for statistical significance using ANOVA followed by Newmans–Keul multiple comparison test. $p<0.05$ implied significance.

RESULTS

Chronic administration of alcohol to the animals of G2 resulted in abnormal rise ($p<0.001$) in the level of total cholesterol and triglycerides. Simultaneous treatment with the EEGL brought down ($p<0.001$) these elevated levels. Likewise the serum level of the enzymes AST and ALT were almost doubled ($p<0.001$) following alcohol administration in G2 which were brought down by EEGL ($p<0.001$).

There was a significant increase ($p<0.001$) in the MDA level in G2, which was significantly ($p<0.001$) brought down with EEGL treatment which indicates decrease in lipid peroxidation. The GSH level was found to be decreased ($p<0.001$) with alcohol, which was significantly ($p<0.05$, $p<0.01$) increased with 200 mg/kg and 400 mg/kg of EEGL treatment respectively (Table 1).

DISCUSSION

Alcohol on continuous administration nurtures hyperlipidemia which will also result in liver damage. Hence enzyme levels were studied to ensure damaging effect of alcohol. This has been confirmed by the elevated AST and ALT levels in G2.

The elevated triglyceride and cholesterol levels noted in G2 following alcohol administration confirms the instigation of hyperlipidemia. Simultaneous administration of EEGL reduces both the elevated lipid levels. So it is inferred that the plant is offering protection against ethanol induced liver damage as well as against hyperlipidemia.

Since enhanced lipid peroxidation has been reported in hyperlipidemia and the extract is protective in hyperlipidemia, the alleged mechanism may be scavenging of free radicals generated during lipid peroxidation.

Table 1 - Effect of EEGL on serum lipids and antioxidant enzymes

Group	AST (U/L)	ALT (U/L)	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	MDA (nmol/100ml)	GSH (mg/100ml)
G1	32.34 ± 6.42	48.64 ± 0.44	58.06 ± 0.52	112.66 ± 0.82	16.84 ± 4.24	72.44 ± 4.82
G2	85.62 ± 4.28 [a]	98.22 ± 4.42 [a]	108.2 ± 4.60 [a]	170.22 ± 1.84 [a]	180.42 ± 6.42 [a]	31.22 ± 5.44[a]
G3	40.68 ± 2.42***	58.46 ± 2.46***	76.64 ± 0.32***	131.48 ± 2.44***	132.28 ± 1.62***	48.24 ± 4.26*
G4	34.52 ± 5.22***	52.38 ± 1.22***	59.66 ± 1.72***	121.82 ± 4.32***	71.22 ± 4.24***	61.24 ± 5.42**

Values are mean ± SEM of 6 animals; ANOVA followed by Newmans – Keul multiple comparison test.
*[a] p< 0.001 compared to G1 (control), *p< 0.05, **p< 0.01, ***p< 0.001 when compared to G2.*
Group 1 (G1) – Normal control.; Group 2 (G2) – Rats treated with 5ml/100 g of 18% ethanol for 45 days.; Group 3 (G3) – Rats treated with 5ml/100 g of ethanol and 200 mg/kg of EEGL for 45 days.; Group 4 (G4) – Rats treated with 5ml/100 g of ethanol and 400 mg/kg of EEGL for 45 days.

There was a 10-fold increase in MDA in G2 following alcohol administration. Glutathione, an antioxidant enzyme was also found to be decreased in G2, which substantiates the increased lipid peroxidation and decrease in antioxidant enzymes following alcohol administration. Simultaneous administration of EEGL decreased MDA and increased glutathione levels which uphold that the free radical scavenging effect and antioxidant property of the plant may be the intended mechanism.

In conclusion, it is evident that *Glinus lotoides* comprehends to be protective in ethanol induced liver damage and hyperlipidemia. These activities could be attributed to the antioxidant and free radical scavenging properties of *Glinus lotoides*. Hence it is worthwhile to isolate the bioactive principle that is responsible for the activity.

ACKNOWLEDGEMENT

We thank Prof.B.G.Desai, Principal, K.L.E.S's College of Pharmacy, and the management, APMC College of Pharmaceutical Education & Research, Himmat Nagar, Gujarat for their encouragement and support throughout this work.

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