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THIOACETAMIDE-INDUCED LIVER TOXICITY IN RATS TREATED WITH *OXALIS STRICTA* LINN EXTRACTS

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ABSTRACT

Aim of present study was to investigate the preliminary screening of aerial part of *Oxalis stricta* extracts for liver Protective activity on experimental rats. Using In-vitro hepatoprotective Model, In this study practical study was started to evaluate anti-hepatic effects of *Oxalis stricta* linn on albino rats. The hepatoprotective effects of Ethanolic Extract of *Oxalis stricta* Linn. (EEOS) towards thioacetamide induced hepatic damage in Albino rats. The extracts of were prepared dose for 250 mg/kg and 500mg/kg against liver toxicity induced by thioacetamide at a dose of thioacetamide 100 mg/kg s.c. once daily. To evaluate some important hepatic parameters such as, Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP) and bilirubin content. Standard drug Silymarin (100 mg/kg) p.o. administered to albino rats was used as reference group. In thioacetamide treated animals shows lipid peroxidation was increased with decrease in superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) levels which represents the hepatic antioxidant status when compared with reference group. It was further confirmed by histopathological observations. From the results it may be concluded that the 70% ethanolic extract of *Oxalis stricta* Linn Possess hepatoprotective activity.

Keywords: *Oxalis stricta*, Liver toxicity, Thioacetamide, Anti-oxidant, Silymarin

INTRODUCTION

Liver is the largest internal organ in the human body, playing a crucial role in digestion, biosynthesis, metabolism and detoxification **[1]**. Liver injury is common metabolic disorder that affect more than 10% the world population. The hepatic injury caused by various pathogenic factors such as, virus, bacteria, parasites, chemical toxins, alcohol and may consequently lead to liver fibrosis, cirrhosis and hepatocellular carcinoma **[2-5]**. Despite the development of modern medicine, hepatic injury is still a worldwide healthy problem. Currently, many drugs for preventing and treating hepatic injury have been developed; unfortunately, the current drugs often have limited efficacy and sometimes with serious side effects when administered chronically or sub-chronically **[6]**.

Oxalis stricta Linn. The literature survey reveals that the plant has been used for stomach cramps, nausea, common cold, to reduce risk of the stroke & fever, Uses in the cooling, to cure scurvy and diuretics, Also used in the diaphoretics and anthelmintic, Used in inhibit absorption of calcium. Oral aid infusion of plant used as wash to refresh the mouth, Used as anti-witch medicine, Relives for thirst and applied for swelling, Used in stimulating appetite, ulcer of mouth and cancer treatment. The different species of *Oxalis stricta* Linn. Contains Oxalic acid, calcium oxalate, potassium. Oxalate, vitamin A and ascorbic acid **[7-9]**.

Thioacetamide acid (TAA), or acetothioamide, is a widely used sulfur containing compound both in the laboratory and in various technical application and can also be present in the environment as organic sulfur compounds **[10]**. It is used to induce an acute liver injury in rats **[11]**.

Thioacetamide-S-oxide binds to macromolecules in a cell that inhibits mitochondrial activity eventually leading to hepatic necrosis **[12]**.

TAA is a hepatotoxic frequently used to induce hepatocellular injury and hepatic fibrosis in rats, and its prolonged administration causes the development of cirrhosis associated with an increased extent of lipid peroxidation **[13]**.

Scientists and researchers study the medicinal value of aerial plant *Oxalis stricta* linn may be due to its constituents that produce a definite physiological action. Therefore, this study seeks to evaluate the Hepatoprotective activity of crude whole plant ethanolic extract of *O. stricta* Linn in thioacetamide induced liver toxicity in rat.

MATERIAL AND METHODS

Chemical Substances

Commercial kits of SGPT, SGOT, Direct bilirubin and Total bilirubin were purchased from Accucare Company (Omaha, NE) and ALP was purchased from span diagnostic (France). Chemical substances including hydrogen peroxide, methanol, thiobarbituric acid, bovine serum albumin, thioacetamide, ferric sulfate, ferric chloride, sodium acetate, and butanol were used.

Plants Collection

Oxalis stricta Linn plants were collected from Botanical Garden of S.C.S College of pharmacy, Harapanahalli, Davangere district of Karnataka. Which was done using valid identification keys by the herbarium experts Prof. K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli, sample were prepared for extraction.

Preparation of Extraction

The aerial parts of the plant were dried separately in shadow at the room temperature then powdered using a mortar. The dried powder (150 gm) obtained is subjected to successive Soxhlet extraction with the solvents with increasing order of polarity i.e. Petroleum ether (60-80° C), chloroform (59.5-61.5° C), ethanol (64.5-65.5° C) and water. The sufficient amount extracts were produced following through extraction using each solvents. Until enough extracts were collected, this extraction procedure was repeated. After completion the extraction procedure, the solvent was entirely evaporated using a vacuum evaporator while operating at reduced pressure, yielding completely dried extract for subsequent use and store dried extract to proper place.

In this study, 70% ethanol (hydro-alcoholic extract) extract, which is used for biological investigations and in vitro and in vivo antioxidant studies, after subjecting it to Studies of phytochemical screening begin with a qualitative chemical test of various chemical components utilizing various chemical reagents **[14, 15]**.

The design of the study

Selection of Experimental Animals

In this study, Albino rats weighing 150-200g and albino mice weighing 20-25g of either sex were used. They were procured from Sri Venkateshwara Enterprises, Bangalore. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C under 12 hour dark / light cycle. They were fed with standard rat feed (Gold Mohur Lipton India Ltd.) and water ad libitum is provided. The litter in the cages is renewed

thrice a week to ensure hygiene condition and maximum comfort for animals.

Ethical clearance for handling the animals is obtained from the Institutional animal ethical committee approved (Reg. no. 157/99/CPCSEA) at SCS college of pharmacy, prior to the beginning of the project work.

Grouping of the rats [16]

30 rats were randomly assigned to 5 groups each consisting of 6 rats.

In the dose response experiment, albino rats were randomly assigned into 5 groups of 6 individuals each.

Group-I - Negative control (received vehicle 1 ml/kg p.o.)

Group-II - Positive control (thioacetamide 100 mg/kg s.c.)

Group-III - Standard drug (Silymarin 100 mg/kg p.o.)

Group-IV - Ethanolic extract of *Oxalis stricta* (250 mg/kg p.o.)

Group-V - Ethanolic extract of *Oxalis stricta* (500 mg/kg p.o.)

On 7th day, 30 min after vehicle, 100 mg/kg silymarin, 70% ethanolic extract 250 mg/kg and 70% ethanolic extract 500 mg/kg of *Oxalis stricta* Linn plants administration to group II, III, IV and V respectively, received thioacetamide (100 mg/kg, s.c) which is prepared in distilled water (2% solution). Food is withdrawn 12 hr. before thioacetamide administration to enhance the acute liver damage in animals of groups II, III, IV and V. The animals were sacrificed 48 hr. after the administration of thioacetamide under mild ether anesthesia.

Collection of blood and tissues from rats

Blood samples of approximately 4 ml were taken from each rat from retro orbital route. Each blood sample was used for the separation of serum after transfer into marked centrifuge tubes. These samples were kept at room temperature for 30 min and centrifuged at 3000g for 10 min. The serum samples obtained in this way were aliquoted for biochemical analyses and were stored at –20C until analysis.

The liver tissues were removed after dissection of rats, washed three times with cold physiological saline and to record the wet liver weight and liver volume. The tissues were sliced into approximately 0.5 – 1 gm using lancet and stored in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group microscopically and GSH estimation and LPO estimation for oxidative study **[15]**.

Evalution of Biochemical markers

Biochemical assays were done for measuring the following parameters. Serum activity of glutamate pyruvate transaminase (SGPT),

Serum glutamate oxaloacetate transaminase (SGOT), Serum alkaline phosphatase (ALP), Serum total bilirubin, Serum direct bilirubin were measured using assay kits. The extent of hepatocyte necrosis was determined with these activities as markers **[17]**.

In vivo anti-oxidant activity

GSH level estimation

Thioacetamide (TAA) induced hepatotoxicity Tissue samples were homogenized in ice cold Trichloroacetic acid (1 gm tissue plus 10 ml 10% TCA) in an ultra turrax tissue homogenizer. Glutathione measurements were performed using a modification of the Ellamn procedure (Aykae, et. al.,) . Briefly, after centrifugation at 3000 rpm for 10 minutes, 0.5 ml supernatant is added to 2 ml of 0.3 M disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium citrate) is added and the absorbance at 412 nm is measured immediately after mixing. % increase in OD is directly proportional to the increase in the levels of Glutathione. Hence, % increase in OD is calculated **[18]**.

In vivo lipid peroxidation

Thioacetamide (TAA) induced hepatotoxicity. The degree of lipid peroxide formation is assayed by monitoring thiobarbituric reactive substance formation. Stock solution of TCA-TBA-HCl reagent: 15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25N hydrochloric acid. This solution may be mildly heated to assist in the dissolution of the thiobarbituric acid. Combine 1.0 ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-0.2 μmol of lipid phosphate) with 2.0 ml of TCA-TBA-HCl and mix thoroughly. The solution is heated for 1 hour in a boiling water bath. After cooling, the flocculent precipitate is removed by centrifugation at 1000 rpm for 2 min. The absorbance of the sample is determined at 535 nm against a blank that contains all the reagents minus the lipid. The malondialdehyde concentration of the sample can be calculated by using an extinction coefficient of 1.56 × 105 M –1 cm–1. **[19]**

Histological studies

The liver sample is dissected out, blotted off blood, washed with saline then stored in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group microscopically **[20]**.

Statistical analysis

All tabulated data were represented as mean ± SD for all statistical analyses. Each group contained six animals. The variation between mean values was determined by one way analysis of variance (ANOVA), using statistical software Graph Pad in Stat version

3.06. P values of < 0.05 were considered as statistically significant.

RESULTS

Phytochemical studies

Preliminary Phytochemical investigation of extracts of the whole plant of *Oxalis stricta* Linn shown that contains alkaloids, proteins and carbohydrates are absent. Moreover extracts were found that glycosides, flavonoids, tannins, are present in ethanolic, aqueous and hydro-alcoholic (30:70). It was qualitatively observed that 70% ethanolic extract contain higher concentration of polyphenolic components and hence selected this extract for further study **(Table 1)**. The screening of phytochemicals' outcomes showed good content.

Toxicity study: Before screening the test extract for the hepatoprotective activity, the extract was subjected to acute toxicity as per OECD guidelines no. 420 (fixed dose method). The extract was found to be toxic at 5000 mg/kg and non-toxic at 2000 mg/kg as indicated by the mortality in the treated group. Hence, the 2500 mg/kg was treated as cutoff tolerable dose, 1/10th (250 mg/kg) and 1/5th (500 mg/kg) of this dose were selected for the further study **[21]**.

Hepatoprotective activity

Different models used for the in vivo screening for Hepatoprotective activity and each model have specific importance and by releasing inflammatory chemical mediators, several chemical induced inflammation. Present study consists of preliminary screening of various extracts of *Oxalis stricta* Linn (aerial parts) for liver protective effects on experimental animals**.**

Effects of EEOS on liver biomarkers

A Significance reduction in liver biomarkers were observed in Silymarin (100 mg/kg), EEOS 100 mg/kg and EEOS 200 mg/kg treated group as compared to model control group (**Table 2 and Figure 1, 2, 3)**.

Effect of EEOS on tissue GSH level

There was a marked depletion of GSH levels in thioacetamide treated group. Treatment with 100 mg/kg silymarin prevented the depletion of GSH to the extent of 90.47%. Treatment with 70% ethanolic extract brought back the decreased GSH levels in a dose dependent manner to a near normal level. The test extract was found to be statistically significant at both lower and higher doses in normalizing tissue GSH levels. However, test extract at 500 mg/kg (highest dose studied) was found to be less effective than Silymarin as standard drug (**Table 3 and Figure 4)**.

Effect of EEOS on tissue in-vivo lipid peroxidation levels

There was a dose dependent inhibition of in vivo lipid peroxidation by both the doses (250 mg/kg and 500 mg/kg) of 70% ethanolic extract. Silymarin (100 mg/kg) has 66.25% inhibition whereas 500 mg/kg 70% EEOS of the bark has 65.20% inhibition. This was statistically significant when compared to the thioacetamide treated group but shown lesser inhibition of lipid peroxidation than the Silymarin treated group (**Table 4 and Figure 5**).

Effects of EEOS on liver weight and liver volume

A significant reduction in weight and volume of liver was observed in Silymarin (100 mg/kg), EEOS 250 mg/kg and EEOS 500 mg/kg treated group as compared to model control group. Moreover, increase in liver weight and liver volume was also significantly prevented by extract or Silymarin treatment group **(Table 5 and Figure 6)**.

Effects of EEOS on Histopathological Changes of experimental animals

In the histopathological examination were performed on the livers of the rats as shown in **Figure 7**. Histology of liver in normal control rat with intact morphology of hepatic globular structure, central vein, portal tract and kupffer cells were normal shown in **Figure 7A**. Diseases induced group observed abnormalities or irregularities such as, extensive fatty change and ballooning of hepatocyte in the livers shown in **Figure 7B**. In Silymarin treated group **showed**, hepatic globular structure, central vein, portal tract and kupffer cells were in normal condition shown in **Figure 7C**. In the case of 250 **mg/kg 70% EEOS treated group showed** there were minimum microvesicle fatty changes near to central vein. Hepatic globular structure, central vein, portal tract and kupffer cells were in normal condition (Slightly inflammation) shown in **Figure 7D**. In the case of 500 mg/kg 70% **EEOS treated group showed** the hepatic architecture was maintained. Areas of kupffer cells proliferation and sinusoids appeared to be normal. There was minimum fatty change without inflammation shown in **Figure 7E**.

Table 1: Phytochemical screening of the different extracts of *Oxalis stricta* extract linn

Types of Phytochemical constituents	Ethanolic Extract	Aqueous extract	70% Ethanolic extract
Alkaloids	–	–	–
Carbohydrates	–	–	–
Flavonoids	+	+	++
Glycosides	++	+	+
Tannins	++	+ +	+++
Protein	–	–	–
Steroids	++	++	+++

***[(+++) Strong Presence, (++): Moderate presence, (+): Presence, (-): Absence]**

Table 2: Biochemical Parameters of liver

Treatment	Biochemical parameters Mean ± SEM				
	SGOT IU/L	SGPT IU/L	ALP IU/L	Total Bilirubin mg/dl	Direct Bilirubin mg/dl
Negative control (1ml dist. water p.o.)	169.16 ± 20.508	109.75 ± 2.491	149.83 ± 10.454	0.90 ± 0.024	0.23 ± 0.030
Thioacetamide (positive control) (1ml dist. Water p.o. + 100mg/kg s.c.)	331.63 ± 15.419	239.2 ± 15.669	464.93 ± 13.887	2.26 ± 0.109	0.57 ± 0.051
Thioacetamide + Silymarin (100mg/kg, s.c. + 100mg/kg, p.o.)	178.954 ± 7.797***	123.7 ± 10.394***	116.28 ± 8.604***	0.92 ± 0.022***	0.29 ± 0.024***
Thioacetamide +70% EEOS (100mg/kg s.c. + 100mg/kg p.o.)	234.33 ± 11.198*	187.99 ± 11.271*	268.73 ± 16.768***	1.33 ± 0.032**	0.42 ± 0.031*
Thioacetamide +70% EEOS (100mg/kg s.c. + 200mg/kg p.o.)	201.53 ± 17.126***	143.1 ± 7.226***	253.61 ± 9.821***	1.00 ± 0.048***	0.38 ± 0.023**

n = 6 per group, tabulated data represents as Mean ± S.D. *p < 0.50, *p < 0.01, ***P<0.001 when comparing treated group with control group

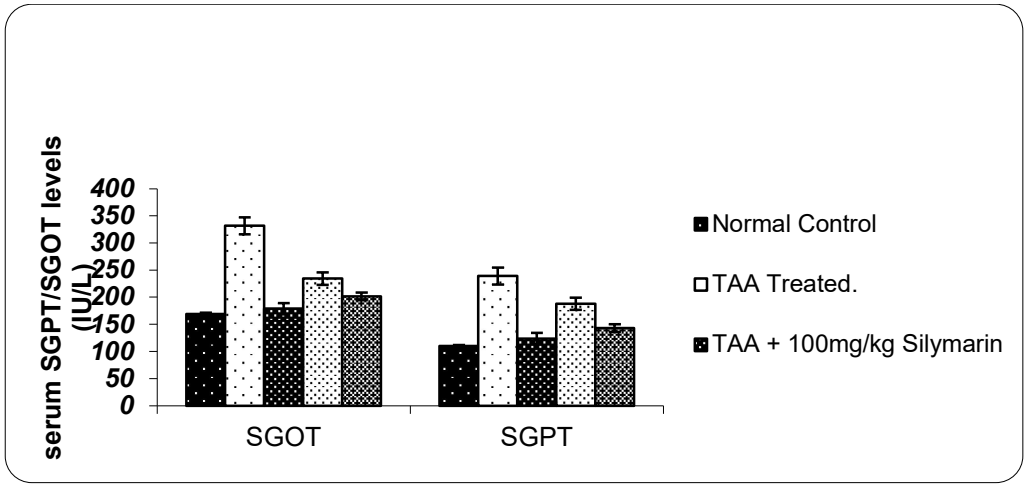


Figure 1: Effect of 70% ethanolic extract of *Oxalis stricta* on total SGOT and SGPT level

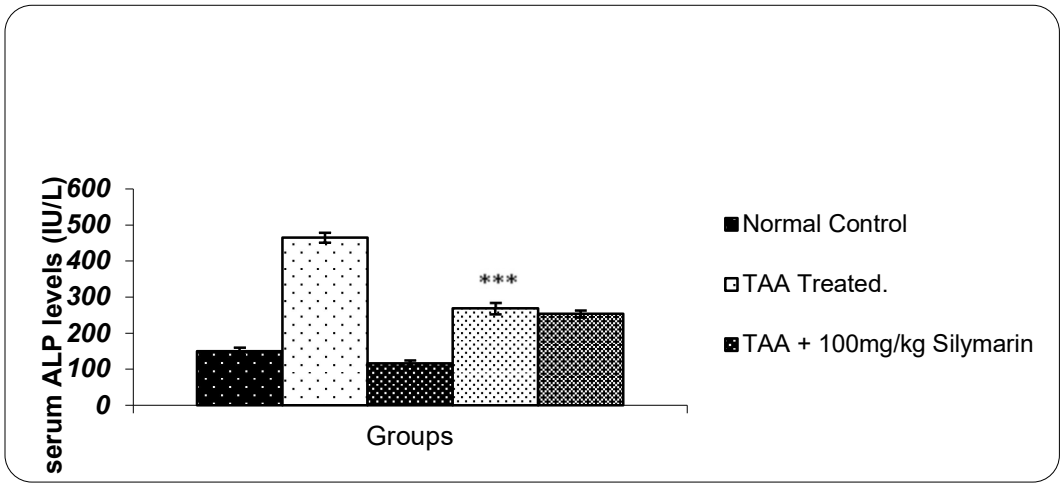


Figure 2: Effect of 70% ethanolic extract of *Oxalis stricta* on ALP level

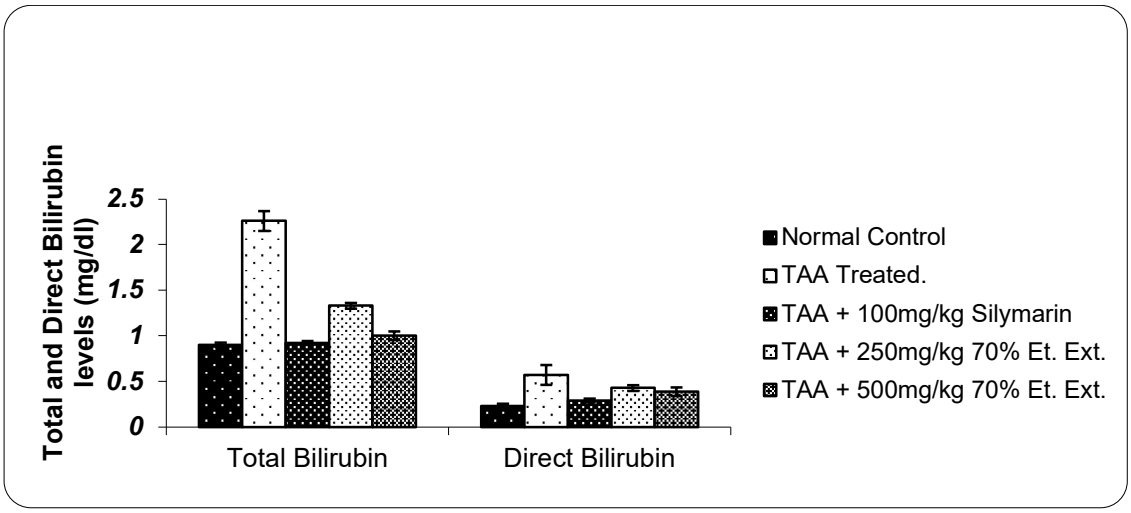


Figure 3: Effect of 70% ethanolic extract of *Oxalis stricta* on total bilirubin/direct bilirubin level

Table 3: Effect of 70% EEOS on tissue GSH levels

Treatment	Absorbance Mean ± SEM	% Increase
Negative control (1ml dist. Water p.o.)	0.93 ± 0.049	--
TAA (Positive control) (100ml/kg s.c. + 100 mg/kg s.c.)	0.42 ± 0.017	--
TAA + Silymarin (100ml/kg s.c. + 100 mg/kg, p.o.)	0.80 ± 0.020***	90.47
TAA + 70% EEOS (100mg/kg s.c. + 250mg/kg p.o.)	0.59 ± 0.026***	40.47
TAA + 70% EEOS (100mg/kg s.c. + 500mg/kg p.o.)	0.73 ± 0.032***	73.80

Values are the mean ± S.E.M. of six rats/treatment. Significance *** P<0.001, compared to Thioacetamide treatment

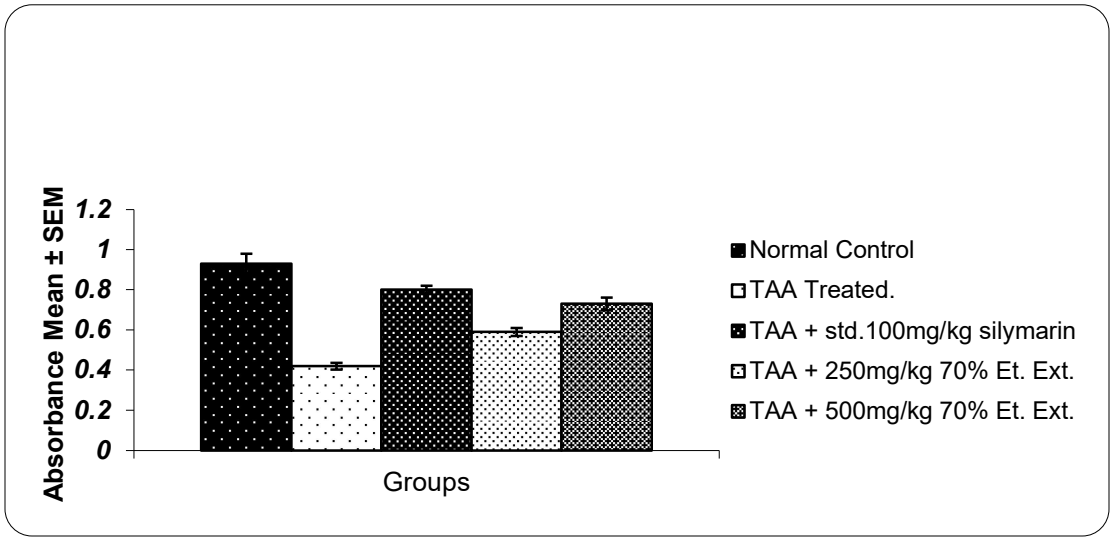


Figure 4: Effect of 70% ethanolic extract of *Oxalis stricta* on tissues GSH level

Table 4: Effect of 70% EEOS on in vivo lipid peroxidation level

Treatment	Absorbance Mean ± SEM	% Inhibition
Negative Control (1ml distilled water)	0.263 ± 0.025	--
Positive Control Thioacetamide (100 mg/kg s.c.)	0.572 ± 0.041	--
Thioacetamide + Standard (Silymarin) (100 mg/kg s.c. + 100 mg/kg p.o.)	0.193 ± 0.019***	66.25
Thioacetamide + 70% EEOS (100 mg/kg s.c. + 250 mg/kg p.o.)	0.283 ± 0.028***	50.52
Thioacetamide + 70% EEOS (100 mg/kg s.c. + 500 mg/kg p.o.)	0.199 ± 0.030***	65.20

Values are the mean ± S.E.M. of six rats/treatment, when Significance ***P<0.001, compared to Thioacetamide treatment

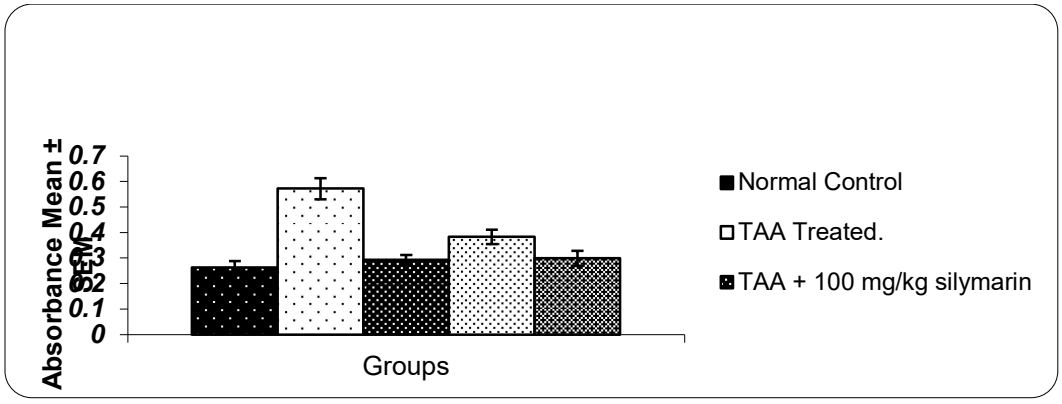


Figure 5: Effect of 70% ethanolic extract of *Oxalis stricta* on tissue LPO levels

Table 5: Effects of EEOS on liver weight and liver volume

Treatment	Liver	
	Volume (ml/100g)	Weight (g/100g)
Negative control (1ml dist. water p.o.)	6.57 ± 0.514	6.43 ± 0.507
Thioacetamide (positive control) (1ml dist. Water p.o. + 100mg/kg s.c.)	6.72 ± 0.311	6.78 ± 0.299
Thioacetamide + Silymarin (100mg/kg, s.c. + 100mg/kg, p.o.)	6.13 ± 0.284***	6.00 ± 0.285***
Thioacetamide +70% ethanolic extract (100mg/kg s.c. + 250 mg/kg p.o.)	6.59 ± 0.309*	6.48 ± 0.313***
Thioacetamide +70% ethanolic extract (100mg/kg s.c. + 500 mg/kg p.o.)	6.38 ± 0.363**	6.30 ± 0.369***

Values are the mean ± S.E.M. of six rats/treatment, when Significance *p< 0.05, **p< 0.01, ***P<0.001, compared to Thioacetamide treatment

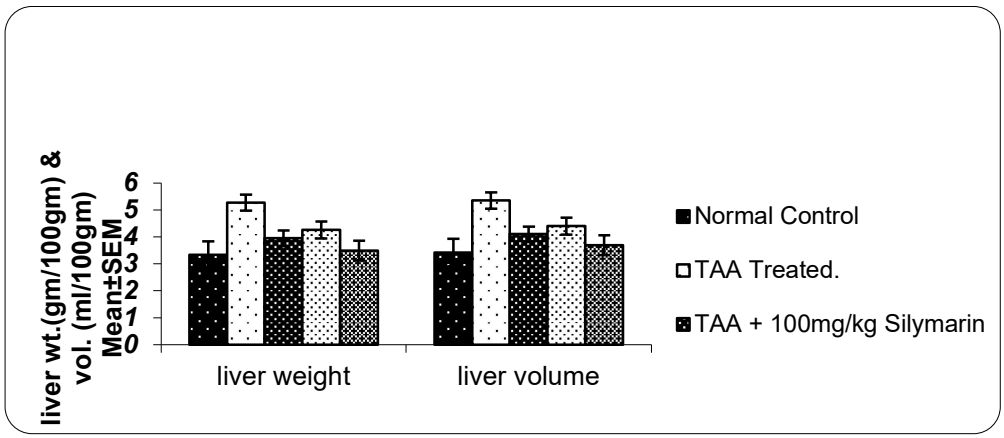
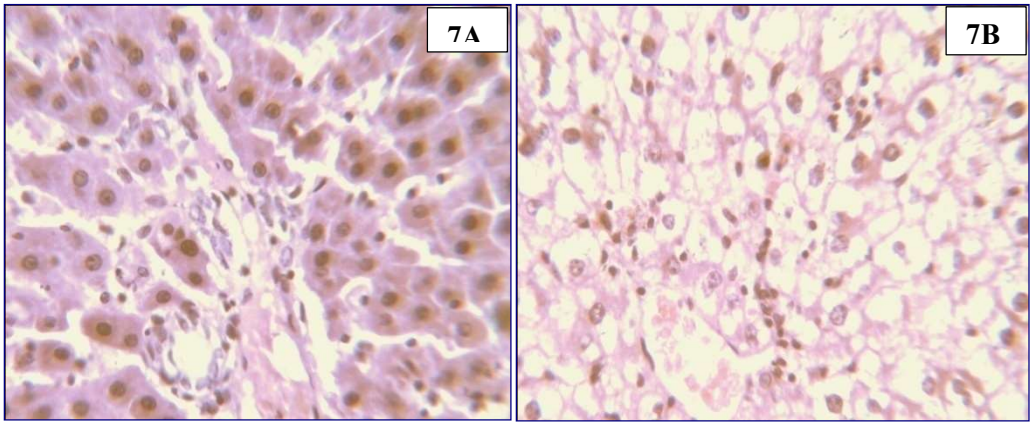
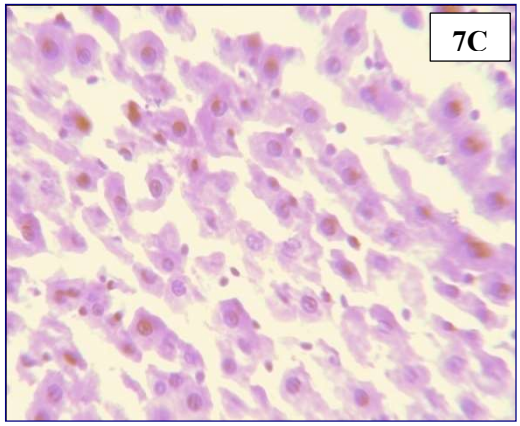


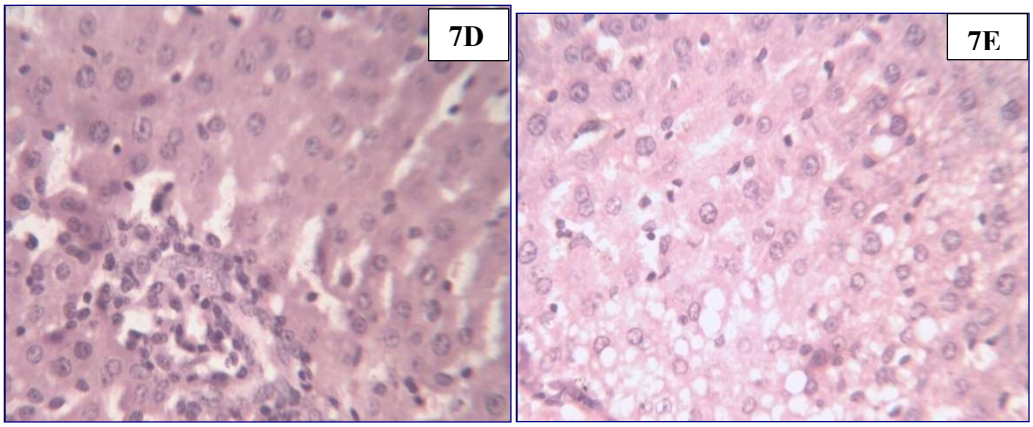
Figure 6: Effects of 70% EEOS on liver weight and liver volume



Liver architecture of Normal Control **Liver architecture of TAA treatment**



Liver architecture of TAA + 100 mg/kg Silymarin treatment



Liver architecture of TAA + 250 mg/kg of 70% EEOS **Liver architecture of TAA + 500mg/kg of 70% EEOS**

Figure 7: Histopathological investigation of liver

DISCUSSION

Liver has magical role in metabolism and detoxification. Many intermediate and end products are created as a result of exposure to various endogenous and xenobiotic substances. These compounds have the potential to cause hepatocellular mortality and are the main causes of liver disease **[22]**.

To need the being maintain the function of the liver, the conventional treatment focus on symptoms management and liver transplantation in severe cases of liver disease. However, there are currently no effective medications that can boost the liver's capacity for detoxification. As a result, research on and application of hepatoprotective medicines derived from plants are growing significantly. Therefore, proving the plant extracts' efficacy in the face of chemically induced hepatotoxicity would be crucial **[23]**.

Thioacetamide (TAA), a potent hepatotoxic agent, is the most widely used criterion for evaluating the hepatoprotective activity of plant extracts. Rats were treated with Ethanolic extract of *Oxalis stricta* linn with different dose and post-administration of thioacetamide **[24]**.

Thus, in this study, rats administered with Thioacetamide resulted significant changes in liver volume and liver weight in the different experimental groups.

On the other hand, rat pre- and post-treated with 70% ethanol extract fraction showed no significant difference in liver volume and liver weight of rat as compared to the negative control group.

TAA-induced hepatotoxicity is used to evaluate the hepatoprotective potential of plant extracts in several animal models. TAA is reductively bioactivated by cytochrome P450 into highly unstable reactive free radicals **[25, 26]**. These may cause cellular damage via peroxidation of membrane lipids and covalently bind with other macromolecules within hepatocytes **[27]**. Membrane damage results in the release of both cytosolic and endoplasmic enzymes, which show the presence of damage in liver structure and function **[28, 29]**.

These are manifested as elevation in the levels of serum liver biomarkers such as, SGOT, SGPT, ALP, total bilirubin and direct bilirubin are important criteria for the evaluation of liver toxicity. The amounts of enzymes that leak into the blood stream indicate the severity of hepatic damage **[30]**.

In the present study, the 70% ethanol extract showed a reduction in the levels of SGOT, SGPT, ALP, total bilirubin and direct bilirubin in a dose dependent manner. The

70% ethanol extract did not produce a visible effect in all biomarkers of hepatic injury in its lower dose, but medium and higher doses were able to produce a significant reduction in the levels of SGOT, SGPT, ALP, total bilirubin and direct bilirubin.

However, *O. Stricta* exhibited hepatoprotective effects to restore the altered serum liver parameters comparable to the effects of silymarin. The percent reduction of biomarkers of liver injury showed that 250 mg/kg and 500 mg/kg of the hydro-ethanolic extract exerted a nearly similar effect as that of the standard.

Pre- and post-treatment with 70% ethanol extract at the two doses (250 mg/kg and 500 mg/kg), largely modulated the severity of TAA- induced liver damage. Enzyme levels' return to near normal levels in 70% ethanol pre- and post-treated rat shows that 70% ethanol extract can stabilize liver cell membranes and prevent the leakage of enzymes. Preventing the production of free radicals and neutralizing them as well as the protection potential of this plant against hepatotoxins can be other probable reasons for the healing effect of areal part of *Oxalis stricta* linn extract.

This collectively suggests that ingredients of the plant responsible for hepatoprotective effect probably are semi-polar and better fractionated by ethanol than the other solvents used. These biochemical effects of the crude 70% ethanol extract of the leaves of *Oxalis stricta* linn were supported by the results of histopathological examination, as evidenced by a decrease in the incidence and severity of histopathological abnormalities or irregularities such as, extensive fatty change and ballooning of hepatocyte in the livers.

The active principle(s) responsible for the hepatoprotective activity of the 70% ethanol extract of *Oxalis stricta* linn. It is not identified which compounds are exactly responsible for the antioxidant and hepatoprotective activities. Previous studies showed that flavonoids were found to have antioxidant activity. Preliminary phytochemical analysis was done on the 70% ethanol crude extract and solvent fractions revealed a variety of secondary metabolites that appeared to be differentially distributed across the extract and fractions.

It is reasonable to suggest that the phytochemicals shown in Table 1 may act individually or synergistically to produce the observed hepatoprotective activity of *Oxalis stricta* linn. Possibly, flavonoids, alkaloids and tannin present in the crude extract and ethanol fraction exerted hepatoprotective effect by their antioxidant activity, prevention

of lipid peroxidation, glutathione and damage to cells as such an action has been suggested for some other plants **[31, 32]**.

To sum up, this study provided further evidence that the 70% ethanol extract possessed a comparable hepatoprotective activity with that of the standard drug is Silymarin **[33, 34]**. Results obtained from the solvent fractions revealed that there was a dose-dependent reduction in all biomarkers of liver injury in pre- and post-treatment of ethanolic extract.

Even though the hepatoprotective mechanism of the plant extract is yet not elucidated, the observed antioxidant activity is one of the predicted mechanisms. Above all, the 70% ethanol extract fractions of the areal parts of *Oxalis stricta* linn would be rewarded as safe based on the results of acute oral toxicity study. Moreover, isolation and characterization of novel antioxidants will be done in future studies by using others analytical techniques.

IV. CONCLUSION

The results of serum biochemical markers and histopathological studies in the 70% ethanol extract fraction pre- and post-treated group support the hepatoprotective effect and provide evidence for the traditional use of *Oxalis stricta* linn for treatment of liver disorders. The larger doses of both the crude 70% ethanol extract produced a remarkable hepatoprotective activity, which was comparable to silymarin. The presence of natural antioxidants in the 70% ethanol extract explain the observed hepatoprotective and in vivo antioxidant activities. These suggest that synergy created between the antioxidant activity and intrinsic protective effects of the plant extract underlie attenuation of TAA-induced liver injury.

Conflict of interest

The authors declare that there are no conflicts of interest.

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