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Original Article

Hepatoprotective Activity of Hydroalcholic Extract of Leaves of *Urena Lobata* Plant on Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats

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ABSTRACT

Introduction: Hepatic damage is a common problem in present days due to life style, food habits, consumption of drugs and alcohol. Drugs which are available in market to treat liver impairments are not completely safe and high cost, so there is urging to develop and identify new agents with fewer side effects. Naturally available compounds were proved better with fewer side effects and better in curing ailments, so *Urena lobata* plant is being rich in a wide variety of secondary metabolites; leaves of this plant were studied for the heptoprotective activity against CCl4induced liver damage in rats. AIM AND OBJECTIVE: To prepare the hydro alcoholic extract of leaf powder and screening for liver protection in albino rats **Materials and methods:** CCL4 induced liver damage in albino rats, where each group had 6 rats received CCl4twice in a week for 4 weeks; test and standard groups received additionally respective doses of drugs daily for 4 weeks, at the end of the 4 weeks blood collected through retro orbital puncture was analyzed for serum SGPT, SGOT, ALP levels and isolated liver study for histopathology. **Results:** Compared to normal group, positive control group has elevated the SGPT, SGOT, ALP levels and also the tissue damage. Silymarin treated group has significantly reduced ($p<0.001$) all the three parameters compared positive control. 250,500mg/kg doses of test also produced a close hepatoprotective activity compared to silymarin. **Conclusion**: As hepatotoxicity is the raising hassle inside the world because of various reasons. Better drugs with minimum side effects are the want of the hour, U. lobata has produced sizeable hepatoprotective activity and much less side effects, so it is able to be drug of preference in future. *U lobata* contains chemical elements, out of which according to literature surveys, we believe that flavanoids are responsible for protection of the liver damage.

Key Words: Hepato Protective, Carbon Tetrachloride, Sylimarin, Hydro alcoholic Extract

1. INTRODUCTION

Hepatotoxicity, the term used to denote the functional and structural damage of liver which may be due to abuse or misuse of potent medicines and consumption of other substances like alcohol etc.[1] This liver damage impairs the normal metabolism and excretion functions of liver leads to

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inappropriate digestion of nutrients, inactivation of drugs, synthesis and secretion of several proteins and so many functions [2, 3]. The incidence and prevalence of this hepatotoxicity has been rising in developing countries and it is life threatening condition which needs to be addressed immediately with effective medication with less side effects [4, 5]. As a part of it, with already existing knowledge, Ayurveda provides natural traditional medicine system with better results by using plant resources. *Urenalobata*, typically known as Caesar weed [6] or congo jute [7, 8,] belongs to the family malvaceae [12]. Caesar weed is also referred to as hibiscus bur, aramina, pink Chinese burr, bur mallow, grand cousin, cadillo, carrapichodomata, malva, mahot cousin, cousin petit, cousin rouge, jut African, coozemahot, dadangsi, and Mau tofu.

2. MATERIALS & METHODS

2.1. Chemicals & instruments

Carbon tetrachloride (CCl4) (Merck), Assay Kits of Alanine aminotransferase (ALT), Aspartate amino-transferase (AST) & Alkaline Phosphate (ALT) (Coral Clinical systems&Trans Asia Bio-Medicals LTD), Olive oil and Silymarin(Sigma Aldrich) were bought from local vendor. Biochemical analyzer we utilized in our college laboratory manufactured byINKARP ES-100P

2.2. Preparation of the plant powder and extraction

The plant leaves of *Urenalobata* Linn have been collected from the village of Atmakuru, close to our college and dried under shade and then powdered. 100grams of the leaf powder of *Urenalobata* become subjected to soxhlet extraction in 1000mL 1:1 ratio of Hydro alcohol (Ethanol + Water) for 8h.The extract obtained turned into concentrated in a water bath till a constant darkish sticky residue was received. This become similarly oven dried and maintained in a desiccators till a consistent weight was acquired. The dried leaf extract acquired was stored in a tightly stoppered container in a groovy region until required.

2.3. Experimental animals

Albino Wistar rats weighs around 150 to 200grams supplied by Mahaveera Enterprises, Hyderabad have been divided into five groups each of six rats. The animals have been acclimatized in an air conditioned animal house at a temperature of 22 ± 2 °C, relative humidity of 57± 2 % and photo cycle of 12:12 h light and dark. The animals were fed with standardized pellet feed and drinking water ad libitum. All of the experimental procedures were carried out in accordance with the guidelines of the Institutional Animal Ethics committee.

2.4. Statistical analysis:

Numerical facts obtained from the examiner were expressed as the mean value ± standard errors of mean. Differences amongst means of manipulate and treated groups have been decided using statistical package deal (Graph prism 5).

2.5. CCL_4 induced hepatotoxicity in swiss albino rats [9,10]

2.5.1. Procedure:

5 groups of 6 rats, each aretreated orally twice a weekwith1mg/kg carbon tetrachloride, dissolved in olive oil 1: 1, over duration of four weeks.

2.5.2. Experimental groupings

- Group I served as control and received olive oil two times a week for a span of four weeks.
- Group II served as positive control and received mixture of CCL4 and olive oil twice every week for 4 weeks.
- Group IIIserved as standard and received Silymarin every day and received mixture of CCL4 and olive oil two times every week for 4 weeks.
- Group IVand GroupV served as test animals and received mixture of CCL4 and olive oil two times per week for 4 weeks and additionally received extract every day of dose 250mg/kg and 500mg/kg respectively.

2.6. Biochemical analysis:

Blood amassed from the animals through retro orbital puncture had been placed into sterile bottles and centrifuged at a rate of 12,000 revolutions/min (rpm) for 10 min. The clean serum acquired was analyzed for Aspartate Transaminase (AST), Alanine Transaminase (ALT), alkaline phosphatase (ALP).

2.6.1. Measurement of SGPT, SGOT &ALP:

2.6.2. SGPT (ALAT) kit

2.6.2.1. Principle:

SGPT (ALAT) catalyzes the transfer of amino group between L-Alanine and α Ketoglutarate to give Pyruvate and Glutamate. The Pyruvate obtained reacts with NADH in the presence of Lactade Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance at 340nm that's proportional to the SGPT (ALAT) activity in the sample.

2.6.2.2. Procedure:

For sample begin assays a single reagent is needed. Combined the contents of 1 bottle of L2 (Starter reagent) into 1 bottle of L1 (Enzyme Reagent). This running reagent is solid for at least three weeks when stored at 2-8^0 C. Rather for flexibility as a good deal of operating reagent can be made as and while desired via mixing together four elements of L1(Enzyme Reagent) and 1part of L2may also be used in place of 1ml of the running reagent directly during the assay.

2.6.3. SGOT (ASAT) kit

2.6.3.1. Principle:

SGOT (ASAT) catalyzes the transfer of amino groupbetween L-Aspartate and α Ketoglutarate to give Oxaloacetate. The Oxaloacetate formed reacts with NADH within the presence of Malate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a lower in absorbance at 340nm that's proportional to the SGOT (ASAT) activity in the sample

L-Aspartate +α Ketoglutarate $\xrightarrow{SGOT}$ Oxaloacetate + L-Glutamate

Oxaloacetate + NADH + H^+ $\xrightarrow{MDH}$ Malate +NAD^+

2.6.3.2. Procedure:

For sample begin assays a single reagent was needed. Pour the contents of one bottle of L2 (Starter reagent) into 1 bottle of L1 (Enzyme Reagent). This running reagent is stable for at least three weeks when saved at 2-8^0 C. As an alternative for flexibility as much of working reagent may be made as and when preferred by using blending collectively 4 components of L1 (Enzyme Reagent) and 1part of L2 (Starter Reagent) alternatively zero.8ml of L1 and 0.2ml of L2 may also be used in place of 1ml of the working reagent directly at some stage in the assay.

2.6.4. ALP kit

The approach in keeping with IFCC (International Federation of Clinical Chemistry) recommendation. This method utilizes 4-nitrophenyl phosphate because the substrate. Under optimized situations ALP present in the sample catalyzes the following reaction

AMP+4-NPP+H_2O $\xrightarrow[Mg\ 2\ +Alkaline\ ph.]{}$ 4-nitrophenol+phosphate.

At the pH of the reaction, 4-nitrophenol has an intense yellow color. The reagent also carries a metallic ion buffer device to make certain that superior concentrations of zinc and magnesium are maintained. The metal ion buffer also can chelate other potentially inhibitory ions which may be present. The reaction is monitored through measuring the rate of increase in absorbance at 405 or 415nm which is proportional to the activity of ALP inside the serum.

3. RESULTS

Table 1: Effect of Extract of *Urena Lobata* on CCL_4 Induced Hepatotoxicity

S.No	Group	Drug&Dose	*SGPT/ALT U/L* (*Mean*±SEM)	*SGOT/AST U/L* (*Mean*±SEM)	*ALP U/L* (*Mean*±SEM)
1	Normal	Olive Oil	127.7±0.57	102.1±0.4	199.25±1.2
2	Positive control	Ccl_4 in Olive oil (1:1)	279.2±0.9	275.23±0.7	315.11±2.1
3	Standard	$Ccl_4$50%in Olive oil. Sylimarin 17.5 mg/kg	131±0.8***	106±0.6***	207±0.5***
4	Test Group 1	Ccl_4 50%in Olive oil., extract 250 mg/kg	198.6±0.7*	182.7±0.6**	260±1.6
5	Test Group 2	Ccl_4 50%in Olive oil., extract 500 mg/kg	149.2±0.48***	136.1±1.2***	216±1.9**

Values are Mean ± S.E.M. (n=6 rats per each group).*p<0.05,**p<0.01,***p<0.001.Significantly different from The group treated with ccl_4.,One –way ANOVA with Dennett's multiple comparison test.

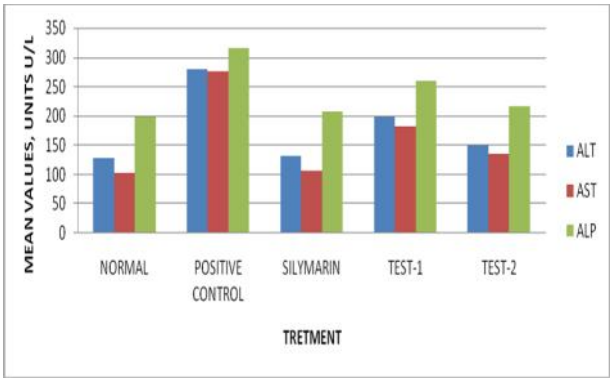

Fig 1: Effect of Extract of *Urena Lobata* on CCL_4 Induced Hepatotoxicity

Values are Mean ± S.E.M. (n=6 rats per each group).*p<0.05,**p<0.01,***p<0.001.Significantly different from the group treated with ccl_4.,One –way ANOVA with Dennett's multiple comparison test.

4. DISCUSSION

To the results provided in Table no.1 and figure No. 1,Positive control group has extensively increased serum SGPT levels compared to normal group, Silymarin treated group significantly reduced ALT.

According SGPT) levels with a P value p<0.001.,250mg/kg & 500mg/kg treated groups also significantly reduced SGPT levels with a P value p<0.05 while compared to the positive control.

Positive control group has significantly increased serum SGOT levels as compared to normal group, Silymarin treated group significantly reduced SGOT levels with a P value p<0.001, 250mg/kg treated group significantly reduced SGOT with a P value p<0.01.500mg/kg treated group also significantly reduced SGOT levels with a P value p<0.001.

Positive control group has drastically increased serum ALP levels in comparison to normal group, Silymarin treated group significantly reduced ALP levels with a P value p<0.001., 250mg/kgtreated group significantly reduced ALP levels with a P value p<0.05., 500mg/kg treated groupdrastically decreased ALP levels with a P value p<0.01.

5. CONCLUSION

As Hepatotoxicity is the raising hassle inside the world because of various reasons. Better drugs with minimum side effects are the want of the hour, *U. lobata* has produced sizeable hepatoprotective activity and much less side effects, so it is able to be drug of preference in future. Moreover, want to set up the mechanism of action of *U.lobata* as hepatoprotective. And in future we do further advanced studies for higher outcomes. *U. lobata* contains numerous lively chemical elements, out of which according to literature surveys on hepatoprotective activity, we believe that flavanoids are responsible for protection of the liver damage triggered through CCL_4.

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