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Protective Effect of *Uvaria narum* Bl. Leaves on Carbon Tetrachloride induced Hepatotoxicity in Rats

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ABSTRACT

In the present study, *Uvaria narum* (Annonaceae) leaves were evaluated for its possible hepatoprotective potential. Elevation in hepatic biomarkers like SGPT, SGOT, ALP, bilirubin & other biochemical parameters like Cholesterol, triglycerides, urea & tissue LPO, where as decrease in total protein, albumin, glucose & tissue GSH, CAT, SOD was observed in CCl_4 induced liver toxicity. Treatment with Silymarin, UNLE-200 and UNLE -400 resorted the altered hepatic biomarkers and biochemical parameters significantly in dose dependent manner. The biochemical observations were supplemented by histopathological examinations of liver sections. Relative organ weights were also reduced dose dependently in extract treated rats. Overall observations indicates that UNLE exerted remarkable hepatoprotective efficacy against CCl_4 induced liver damage were comparable to reference standard Silymarin (100mg/kg) that is, may be due to its augmenting endogenous antioxidant mechanisms. These findings suggest the use of plant for treatment of liver diseases in oriental traditional medicine.

INTRODUCTION

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward and Daly, 1999). Some of the major functions include Carbohydrate, protein, Fat, metabolism, detoxification and secretion of bile. Therefore, the maintenance of a healthy liver is vital to overall health and well being (Tortora and Anagnostakos, 2000).

Liver diseases are posing as a major health problem around the world. Hepatitis, Viral infection, toxic industrial chemicals, alcohol, alfatoxin, water pollutants are the major risk factor of liver diseases. In spite of the tremendous advances in modern medicine, there is no effective drug available that stimulates liver function, offer protection to the liver from damage or help to regenerate hepatic cells (Chattopadhyay, 2003). It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace currently used drugs of doubtful efficacy and safety.

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Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material (WHO, 1993). In absence of a reliable liver protective drug in the modern system of medicine, a number of medicinal preparations in Ayurveda are recommended for the treatment of liver disorders (Karthikeyan and Deepa, 2010). Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for liver diseases. *Uvaria narum* (Annonaceae family) is a medicinal plant widely distributed in foot hills of Western Ghats. Literature survey showed the plant leaves are used to treat liver diseases (Pandey, 2011). No systemic study to assess this activity has been reported hence present investigations were carried out to evaluate the drug for its hepatoprotective activity.

MATERIALS & METHODS

Animals

Wistar strain rats of either sex (150-180g) were procured from inbreed facility of Srinivas college of Pharmacy, mangalore. They were maintained under standard conditions (temperature 22±2°C, relative humidity 50±5% and 12 hour light dark cycle. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet & water *ad libitum*.

The Institutional Animal Ethics Committee approved the Experimental protocol. All the animals received humane care and use of Laboratory Animals" prepared by "National academy of Sciences" and published by the "National Institute of Health".

Plant material and extraction

The fresh leaves of *U.narum* used for the present studies were collected from local areas of Mangalore, in May 2012. It was authenticated by Mr. Dinesh Nayak Advisor (Green belt), Mangalore SEZ Limited.

The leaves were dried under shade, pulverized into coarse powder and were extracted using ethanol as a solvent by using Soxhlet apparatus, until colourless solvent appeared in siphon tube.

Further extract was concentrated by rotary flash evaporator. The extracted was dried & kept in desiccator for further study.

Drugs and Chemicals

All chemicals and solvents used in the study were of analytical grade. The Chemicals inducing hepatotoxicity like CCl_4 (Karnataka fine chem, Bangalore), Nitroblue tetrazolium, Phenazine methasulphate, NADH, Thiobarbituric (Himedia suppliers), all estimation kits were obtained from Agapee distributers.

ACUTE TOXICITY STUDIES

The acute oral toxicity study of was performed as per the OCED guideline No.425. Limit test was performed at dose 2000 mg/kg as per the guideline. Animals were observed after dosing individually at least once during the 30 minutes for 4 hrs, periodically during the first 48 hours and daily thereafter for 14 days for signs of toxicity and mortality, if any.(OCED 425)

CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY

Experimental design

Wistar rats of either sex weighing between 150-200g were divided into five groups of six animals each. For the first nine days of study Group I & II were fed with normal feed & water. Group III animals were treated with Silymarin (100mg/kg) and group IV & V were treated with *U.narum* leaf extract (200mg/kg and 400mg/kg extract was suspended in 1% Gum tragacanth) respectively for 9 days. All the treatment was done post orally.

On 9^{th} day, all the animals except Group I were intoxicated by the administration of CCL_4 1ml/kg i.p. (1:1 of CCl_4 in olive oil). After 48hrs of intoxication by CCL_4 administration, blood was collected through retro orbital puncture and analyzed for various biochemical parameters. Animals were sacrificed using ether anesthesia and liver was dissected out and used for histopathological studies (Roy et al. 2006).

ASSESSMENT OF HEPATOPROTECTIVE ACTIVITY

Biochemical Parameters

The collected blood was used for estimation of serum biochemical parameters like SGOT, SGPT, ALP, total (BILT) & direct (BILD) bilirubin, total proteins (TOT Protein), albumin (ALB), total cholesterol (CHO), triglycerides (TG), urea & glucose contents were estimated by using commercially available reagents kits (AGAPPE) (Chavda et al., 2010), (Kumar et al., 2009).

Liver tissue was estimated for Lipid peroxidation (LPO), reduced glutathione (GSH), catalase (CAT) & superoxide dismutase (SOD) were assayed according the methods described by previous workers and were expressed in Absorbance & % increase or decrease (Oyedemi et al., 2010).

Relative organ weight analysis

Animals were sacrificed, liver, spleen, left lung, heart, Kidney were removed, washed with ice cold saline and were immediately weighed and liver volume was also measured ,all the weights & volume of organs were expressed as g/100g, ml/100g body weight (Murugaian et al., 2008)

Histopathological studies

For histopathological study, the fresh liver tissues were collected and immediately fixed in 10% formalin, dehydrated in gradual ethanol (50-100% v/v), cleared in xylene and embedded in paraffin. Sections (4-5 μm) were prepared and then stained with hematoxylin-eosin dye for photo microscopic observations (Luna, 1998).

STATISTICAL ANALYSIS

All the results are expressed as Mean ± SEM, the results were analysed for statistical significance by one-way ANOVA followed by Dunnett's test. $P<0.05$ was considered as statistical significant.

RESULT & DISCUSSION

Acute toxicity of ethanolic extract of *U.narum* was studied according to OECD No.425 guide line the extract was found to be safe at dose of 2000mg/kg p.o. hence one tenth of 2000mg/kg and one fifth of 2000mg/kg (i.e. 200mg/kg & 400mg/kg) was selected as low dose & high dose for present study.

Since the changes associated with CCl_4 induced liver damage are similar to that of acute viral hepatitis, $CC1_4$-mediated hepatotoxicity was taken here as the experimental model for liver injury (Rubinstein, 1962) The hepatotoxicity induced by CCl_4 is due to its metabolite $CCl_3^{\bullet}$, a free radical that binds to lipoprotein and leads to peroxidation of lipids of endoplasmic reticulum. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective

effects. Protection of hepatic damage caused by carbon tetrachloride administration was observed by recording SGOT, SGPT, ALP and BLN levels because they have been reported to be sensitive indicators of liver. The experimental results showed the activities of hepatospecific enzymes like SGPT, SGOT, ALP & bilirubin were increased in CCl_4 intoxicated animals this is due to the disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membrane (Ramakrishna et al., 2013).

However the increased levels of above enzyme as were significantly reverted by the pretreatment with Silymarin (100mg/kg) & UNLE at different doses (200 & 400mg/kg). (**Table: 1)** Biochemical parameters like total protein, albumin & glucose were depleted conversely triglyceride, cholesterol, urea levels were increase in CCl_4 treated animals this is due to free radical $CCl_3^{\cdot}$ binds to Proteins & lipids in the presence of oxygen to induced lipid peroxidation. Result in loss of metabolic enzyme activation, reduction of protein synthesis & loss of glucose6-phosphate.

Total cholesterol and triglycerides (TG) increases with CCl_4 administration which induces an increase synthesis of fatty acids as well as decreased release of hepatic lipoproteins, the accumulation is due to the interference with formation of TG and secreting mechanism (Rajesh and Latha, 2004). Experiment results showed, prophylactic treatment with Silymarin and UNLE-200 and UNLE-400 significantly altered biochemical parameters which were comparable to normal control. (**Table: 2).** Relative organs weight such as Liver, spleen, kidney, left lung, heart weight & liver vol of rats were elevated in CCl_4 intoxication. Conversely, the relative organ weights were reversed to near normalcy in CCl_4 induced group of rats by silymarin & UNLE (200 & 400mg/kg).**Table:3.**

Hepatoprotective activity is associated with antioxidant activity, since it is free radical mediated damage. Elevated level of MDA reflects an enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms to prevent formation of excessive free radicals. The enzymatic antioxidant defence systems are the natural protector against lipid peroxidation.

SOD, CAT enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage. Earlier studies regarding mechanism of CCl_4 induced hepatotoxicity have shown that GSH plays a key role in detoxifying the reactive toxic metabolites of CCl_4 & that liver necrosis begins when GSH store are markedly in depleted state (Surendran et al. 2011).

CCl_4 exposure to animals depleted stores of antioxidant enzymes and enhanced lipid peroxidation. The experimental changes were significantly reversed in Silymarin and UNLE (200 & 400mg/kg) treated animals **Table: 4**

Histopathological studies, showed CCl_4 to produce extensive disarrangement of normal hepatic cells with centrilobular necrosis. Treatment with silymarin and *U.narum* extract produced mild degenerative changes and mild to moderate centrilobular necrosis when compared with control (**Fig.1**). All these results indicate a hepatoprotective potential of the extract.

Table. 1: Effect of Silymarin and UNLE on Serum SGPT, SGOT, ALP, BILT and BILD in CCl_4 induced liver toxicity.

Groups	Treatment	SGPT (U/l)	SGOT (U/l)	ALP (U/l)	BILT (mg/dl)	BILD (mg/dl)
Normal control	Saline	82.42 ± 3.68	97.84±11.97	376.0±26.53	0.55±0.04	0.19±0.06
Toxic control	CCl_4 1ml/kg	311.9±23.94	297.4±13.20	954.8±18.29	1.69±0.24	0.42±0.03
Standard	Silymarin 100mg/kg	233.6±10.16**	204.5±16.80***	394.0±40.05***	0.51±0.04***	0.13±0.02***
Low dose	UNLE 200mg/kg	258.7±7.17*	223.1±10.66**	444.4±58.80***	0.57±0.02***	0.22±0.02***
High dose	UNLE 400mg/kg	240.9±10.76**	211.4±13.02***	430.5±49.24***	0.53±0.03***	0.21±0.02***

All the values are Mean ± SEM, n=6 ns-p>0.05, *p<0.05, **p<0.01, ***p<0.001 One way ANOVA followed by Dunnett's test compared to toxic control.

Table. 2: Effect of Silymarin and UNLE on TOT Protein, ALB, TG, CHO, Urea and Glucose in CCl_4 induced liver toxicity.

Groups	Treatment	TOTprotein(g/dl)	ALB(g/dl)	TG(mg/dl)	CHO(mg/dl)	Urea (mg/dl)	Glucose(mg/dl)
Normal Control	Saline	8.33±0.44	3.60±0.24	98.50±4.53	135.8±11.67	37.50±2.95	133.7±11.42
Toxic control	$CCl_4$1ml/kg	5.22±0.45	2.26±0.14	214.1±8.80	198.4±8.81	58.50±3.32	58.72±3.67
Standard	Silymarin100mg/kg	8.85±0.32***	3.41±0.13***	149.4±15.54**	117.3±6.34***	33.77±1.34***	105.2±8.86***
Low dose	UNLE200mg/kg	8.35±0.42***	3.08±0.12**	194.9±8.40^{ns}	134.3±10.55***	45.83±2.89**	95.75±8.07**
High dose	UNLE400mg/kg	8.53±0.31***	3.25±0.14***	159.7±10.11**	126.7±9.81***	41.17±1.72***	98.69±5.07**

All the values are Mean ± SEM, n=6 ns p>0.05, *p<0.05, **p<0.01, ***p<0.001 One way ANOVA followed by Dunnett's test compared to toxic control.

Table. 3: Effect of Silymarin and UNLE on Relative organ weight in CCl_4 induced Liver toxicity.

Groups	Treatment	Liver weight g/100g	Liver vol ml/100g	Spleen weight g/100g	Kidney weight g/100g	Left lung weight g/100g	Heart weight g/100g
Normal Control	Saline	3.93±0.25	4.33±0.40	0.32±0.03	0.56±0.03	0.25±0.01	0.29±0.02
Toxic control	CCl_4 1ml/kg	5.18±0.27	5.20±0.29	0.95±0.19	0.86±0.03	0.39±0.02	0.48±0.05
Standard	Silymarin100mg/kg	3.57±0.11***	4.04±0.15**	0.47±0.04**	0.66±0.04**	0.22±0.01***	0.28±0.01**
Low dose	UNLE200mg/kg	3.91±0.04***	4.54±0.22^{ns}	0.66±0.06^{ns}	0.77±0.02^{ns}	0.25±0.01***	0.36±0.03*
High dose	UNLE400mg/kg	3.87±0.06***	4.24±0.11*	0.50±0.05**	0.68±0.03**	0.23±0.04***	0.30±0.01**

All the values are Mean ± SEM, n=6 ns p>0.05, *p<0.05, **p<0.01, ***p<0.001 One way ANOVA followed by Dunnett's test compared to toxic control.

Table. 4: Effect of Silymarin and UNLE on GSH, LPO, SOD and CAT in CCl_4 induced liver toxicity.

Groups	Treatment	GSH Abs at 412nm	LPO Abs at 535 nm	SOD Abs at 560 nm	CAT Abs at 620 nm
Normal control	Saline	0.61 ± 0.04	0.05 ± 0.02	0.87 ±0.03	0.47 ± 0.04
Toxic control	$CCl_4$1ml/kg	0.24 ± 0.03	0.31 ± 0.01	0.08 ± 0.06	0.21 ±0.02
Standard	Silymarin100mg/kg	0.52 ± 0.03 ***(+76.10)	0.14 ± 0.02***(-55.64)	0.68 ± 0.03***(+87.66)	0.40 ±0.02 ***(+87.76)
Low dose	UNLE200mg/kg	0.43 ± 0.02^{ns}(+45.12)	0.22 ±0.02**(-29.75)	0.45±0.07***(+81.33)	0.33 ± 0.01 **(+52.84)
High dose	UNLE400mg/kg	0.49 ±0.02**(+65.57)	0.20 ±0.01**(-34.67)	0.59 ± 0.06***(+85.82)	0.37 ± 0.01***(+73.53)

All the values are Mean ± SEM, % increase (+) or decrease (-) is shown in parentheses, n=6 ns-p>0.05, **p<0.01, ***p<0.001, One way ANOVA followed by Dunnett's test compared to toxic control.

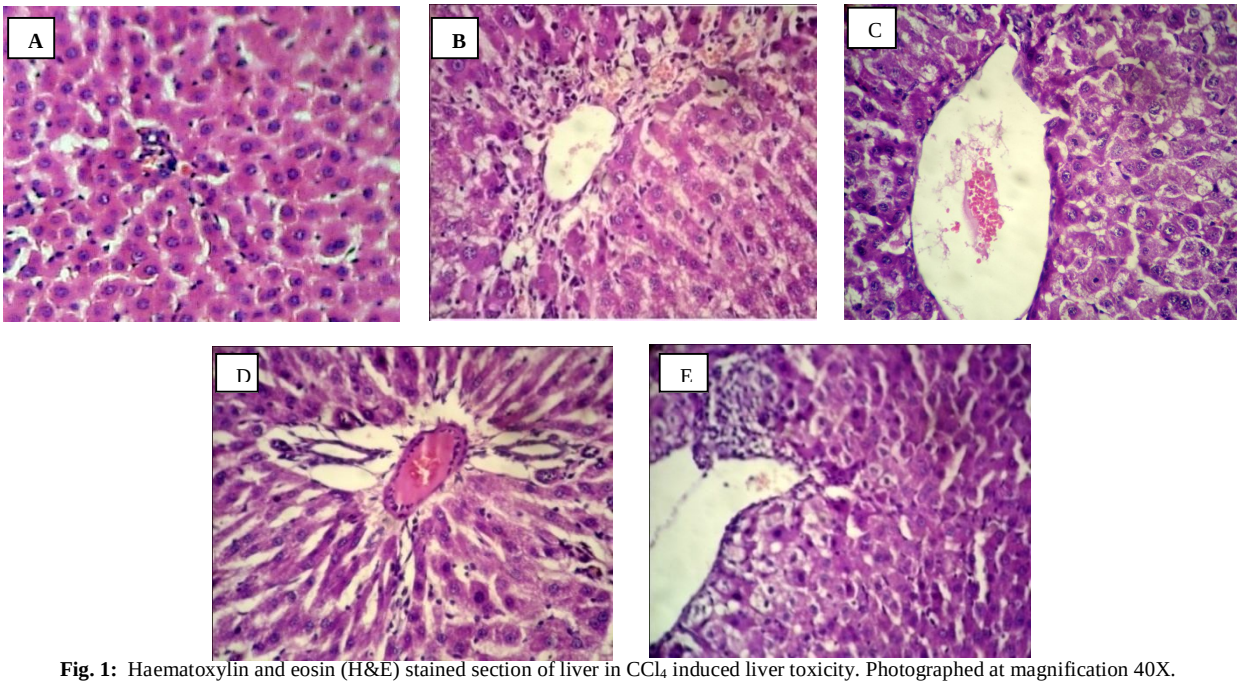

Fig. 1: Haematoxylin and eosin (H&E) stained section of liver in CCl_4 induced liver toxicity. Photographed at magnification 40X. **A -** Liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central veins. **B -** Disarrangement of normal hepatic cells with centrilobular necrosis, were observed in CCl_4 intoxicated animals. **C, D, E -** The liver sections of the CCl_4 induced hepatotoxic rats treated with Silymarin 100mg/kg and UNLE at the dose level of 200 & 400 mg/kg b.w showed mild to moderate focal necrosis. The architecture of UNLE treated group was comparable with standard silymarin.

CONCLUSION

From present investigation, it is concluded that ethanolic extract of leaves of *U.narum* possess significant hepatoprotective activity against CCl_4 induced liver toxicity in rats. This may be due to presence of reported phytoconstituents and specially flavonoids which possess antioxidant activity. Further research, to isolate hepatoprotective principle & exact mechanism involved, is needed.

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