Hepatoprotective Action of *Holoptelea integrifolia* Planch. Against Paracetamol induced Hepatotoxicity

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Abstract **:** The present study was conducted to evaluate the hepatoprotective action extract of ***Holoptelea integrifolia*** against paracetamol induced liver damage in rats. To evaluate the hepatoprotective action of *Holoptelea integrifolia* Wistar albino male rats weighing between 150-200 gm were used. Rats were divided into six groups (each contains six animals). Group 1 served as normal control received 1% tween 80 (1ml/kg). Group 2 served as Hepatotoxic control received paracetamol 2g/kg body weight. Group 3 served as standard control received silymarin 100mg/kg and paracetamol on 3rd day and Group 4,5 and 6 received methanolic extract of *Holoptelea integrifolia*150mg/kg, 300mg/kg and 450 mg/kg respectively and paracetamol on 3rd day. At the end on day 5th blood sample was collected by heart puncturing method for the estimation of markers like ALT, AST, ALP, Total bilirubin and total protein. Liver was isolated for histopathological estimation. From the result it was concluded that methanolic extract of *Holoptelea integrifolia* at the dose of 450mg/kg is effective and shows significant decrease in the level of ALT, AST, ALP, total bilirubin.

Index Terms **: -** Hepatoprotective, *Holoptelea integrifolia*, Histopathology, Paracetamol, Silymarin.

Abbreviations **: -** ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphate; g, gram; mg, milligram; kg, kilogram; CPCSEA, Committee for the purpose of control and supervision of experiments on animals; OECD, Organisation for economic co- operation and development; ºC, degree Celsius; w/w, weight/weight; v/v, volume/volume; SEM, Standard error of mean; ANOVA, Analysis of variance; P, Probability; U/L, units/litre; dl, decilitre; FIG, Figure.

1. INTRODUCTION

Holopteleaintegrifolia is an ornamental plant distributed in India, China, Burma and Srilanka. In India it is distributed in sub Himalayan region of Assam, Bihar, Ajmer, Bundelkhand& used by local community for treatment of diseases. (Prajapati D *et al*.,2010).Plant contains various pharmacological activities like anti-inflammatory, digestive, carminative, anti-ulcer, anti-bacterial-, anti-fungal, antioxidant, and wound healing properties. Stem and bark of the plant is used Anti-bacterial, Antifungal, and Antioxidant activities (Showkhat AG *et al.,* 2014).Hydro-alcoholic extract of the leaves and bark is used as hypolipidemic agent(Subhash AK *et al.,* 2013). Plant contains various Phyto-chemical constituents like terpenoids, sterols, saponins, tannins, proteins, carbohydrates, and alkaloids (Ahmad S *et al.,* 2012). Plant contains a rich amount of flavonoids, phenol (Kumar RS *et al.,* 2013).

Liver is the largest gland of our body and is a vital organ which perform major functions in metabolism and excretion (Meganathan M *et al.*,2011). Paracetamol hepatotoxicity is very common caused by reactive metabolite i.e. N-acetyl-p-benzo quinoneimine (NAPQI) induces oxidative stress(Shah VN *et al.,* 2011).

Present study aimed at hepatoprotective action of *Holoptelea integrifolia* against paracetamol hepatotoxicity in male Wistar albino rats.

2. MATERIAL AND METHODS: -

2.1 Drugs and Chemicals

Paracetamol was used to cause hepatotoxicity in the animals and silymarin was used as a standard drug for the comparison with test drug.

2.2 Plant material

The stem of Holoptelea integrifolia were collected in the month of April and May and the specimen was identified by Dr. G.P. Sinha Scientist, Botanical Survey of India Central Regional Centre, 10 Chatham Lines, Allahabad, 211002.

Specimen with Voucher number SIP/2017/244 has been deposited at the Botanical Survey of India.

2.3 Experimental animals

Male albino Wistar rats weighing 150- 180 g were procured from NIN, Hyderabad, India.the animals were placed in the propylene cages with paddy husk bedding at the temperature of 24±2ºC and relative humidity 30-70%. 12:12 hours light day cycle was maintained and rats were provided standard pellet diet. All the experimental procedure and protocols were authenticated by Animal Ethical Committee according to the guidelines of CPCSEA.

2.4 Acute oral toxicity

Acute oral toxicity testing was carried out as per the guidelines of OECD, revised draft guidelines 423, received from CPCSEA, Ministry of social justice and empowerment, Govt of India (OECD, 2005).

2.5 Preparation of Plant Extracts

The stem of Holoptelea integrifolia were shade dried and then powdered by mechanical grinder. The coarse powder was defatted by petroleum ether and extracted by methanol using cold maceration process. The

extract was concentrated by rotatory evaporator under reduced pressure. Suspension of concentrated extract was prepared by dissolving in 1% tween 80 solution and phytochemical screening was carried out.

2.6 Determination of extractive value

Extractive value of stem of Holoptelea integrifolia in various solvents were observed by using formula: -

$$\text{EXTRACTIVE VALUE} = \frac{\text{INITIAL WEIGHT} - \text{FINAL WEIGHT}}{\text{FINAL WEIGHT}} \times 100$$

SOLVENTS	METHANOL	WATER	ETHANOL	CHLOROFORM	PETROLEUM ETHER
% YEILD	17.6	15.9	12.4	6.6	5.8

Highest percentage yield was found in methanolic extract i.e. 17.6% w/w as compared to other solvents.

2.7 Determination of ash values: -

1.Total Ash Value

5 grams of dried and powdered plant material was taken in the pre-weighed clean sintered silica crucibles. Then, they were incinerated by gradual increasing of the temperature (400-500 °C) in the muffle furnace till white ash and constant weight of ash obtained. The crucible was cooled to room temperature in a desecrator and weighed the ash and calculated the % of total ash with reference to the air-dried sample of the crude drug using following formula:

$$\text{Total ash value (\%)} = \frac{Z-X}{Y} \times 100$$

Drug	Weight of sample(Y)	Weight of crucible (X) in gm	Weight of crucible with ash (Z) in gm	Total ash (%)
Stem	5 gm	31.966	32.405	8.78

2.Acid Insoluble Ash Value

The total ash content of the plant material obtained was boiled for 15min, after adding 25ml of 25 %(v/v) HCl in to a 100 ml beaker and was allowed to cool. It was filtered through a Whatman filter paper No. 44 (ash less) and wash the residue twice with hot water. The insoluble ash thus retained on filter paper along with paper was ignited in a pre-weighed sintered crucible (1000 ºC). Then the crucible along with the residue was weighed and calculated the acid insoluble ash content using the following formula: -

Acid insoluble ash value = (M0-M1÷M2) × 100

Drug	Weight of crucible + ash(gm) [M0]	Weight of empty crucible (gm) [M1]	Sample weight (gm)[M2]	Acid insoluble ash (%)
Stem	32.112	31.966	5	2.92

3. Water soluble ash value

The total ash value was determined using 5 g of the air-dried powdered sample. The total ash was boiled for 5 minutes with 25 ml of distilled water; the insoluble matter was collected on an ash less filter paper, washed with hot distilled water, and ignited for 15 minutes at a temperature not exceeding 450 ºC. The weight of the insoluble matter was subtracted from the weight of the total ash; the difference in weight represents the water-soluble ash. The percentage of the water-soluble ash was calculated with reference to the airdried powdered plant sample. It was calculated by using following formula: -

Water Soluble Ash value = Total Ash value -Water insoluble ash value

water soluble ash value was found to be 3.29%.

Phytochemical Evaluation

S. NO.	Name of Test	Chemical Constituents	Petrole-um Ether	Methanol	Ethanol	Distilled water	Chloroform
1.	Fehling soln.	Reducing Sugar	+	+	-	-	-
2.	Xanthoprotein	Protein	+	-	+	+	-
3.	5% Fecl3 soln.	Phenols	-	+	-	+	-
4.	Dragendroff's test Mayer's test	Alkaloids	+ +	+ +	+ +	+ -	- +
5.	Salkwoski test	Steroids	+	+	+	-	+

6.	Lead Acetate Test Alkaline reagent	Flavonoids	- +	+ +	+ +	- +	+ +
7.	Killer Killani Test	Glycoside	+	+	+	+	+
8.	Liberman Burchard test	Tri Terpenoids	-	+	-	+	-
9.	Ferric chloride test	Tannin	+	+	+	+	-
10.	Foam Test	Saponin	-	-	-	+	+

2.8 Hepatoprotective activity

Animals were divided into 6 groups each comprising of 6 animals. Group I- served as normal control received 1% tween-80 (1 mg/ml) for 4 days. Group II- served as Hepatotoxic control received paracetamol (2g/kg) on Day 3rd. Group III- served as standard control received single dose Silymarin (100mg/kg) for three consecutive days. Group IV and V and VIreceived extract of *Holoptelea integrifolia*at the dose of 150mg/kg, 300mg/kg and 450mg/kg respectively for 3 days and Group II, III, IV, V and VI received paracetamol (2g/kg) on 3rd day. All test drug and standard drug were administered orally. After 48 hours blood sample was collected by heart puncturing method and serum was subjected for estimation of ALT, AST, ALP, Total protein and Total bilirubin.

3. STATISTICAL ANALYSIS

Data were represented as ±SEM and result were analysed by one-way ANOVA followed by Dunnett's multiple comparison test using Graph pad instat 3.0 software. P values <0.01 were considered significant.

4. RESULTS

The result of hepatoprotective activity of *Holoptelea integrifolia* is shown in Table 1. Which shows that after the administration of plant extract causes significant reduction the level of ALP, AST, AST, Total Protein and Total Bilirubin when compared to Paracetamol.

S.NO.	Treatment	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	Total Bilirubin	Total Protein

					(mg/dl)	(gm/dl)
1.	**Normal control**	**56.6±0.347**	**172.7±0.265**	**253.9±0.543**	**0.92±0.042**	**9.3±0.234**
2.	**Paracetamol 2g/kg**	**132±0.229**	**234.7±0.388**	**390.5±0.347**	**4.7±0.248**	**17.82±0.411**
3.	**Silymarin 100 mg/kg + paracetamol**	**28.7±183**	**90.0±0.250**	**144.0±0.451**	**0.47±0.013**	**5.537±0.197**
4.	**Methanolic extract of Holoptelea integrifolia 150 mg/kg + Paracetamol**	**52±0.290**	**103.5±0.471**	**168.5±0.271**	**0.84±0.020**	**7.355±0.224**
5.	**Methanolic extract of Holoptelea integrifolia 300 mg/kg + Paracetamol**	**28.9±0.350**	**89.8±0.157**	**149.5±0.281**	**0.55±0.023**	**6.067±0.171**
6.	**Methanolic extract of Holoptelea integrifolia 450 mg/kg + Paracetamol**	**21.8±0.386**	**75.7±0.213**	**129.7±0.242**	**0.26±0.025**	**4.95±0.263**

5. HISTOPATHOLOGICAL RESULTS

Results of histopathological studies confirmed the hepatoprotective action of *Holoptelea integrifolia*.

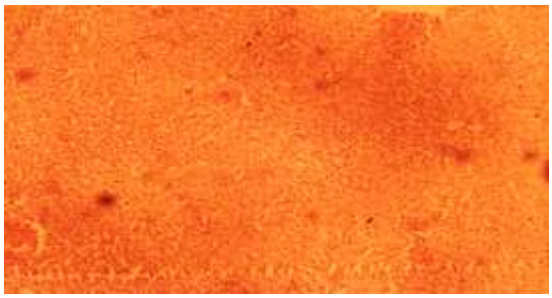

FIG 1: - Normal group showing normal cellular architecture with distinct sinusoidal space and central vein.

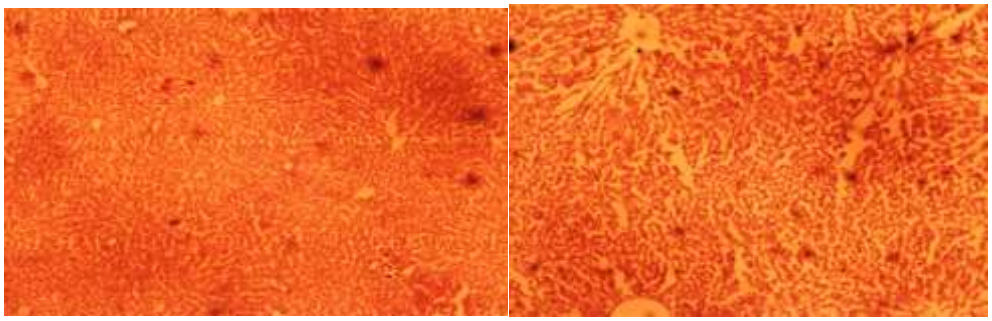

FIG 2: - Standard silymarin (100 mg/kg) Treated group showing less disarrangement of hepatocytes as well as marked regeneration activity.

FIG 3: - Methanolic low dose (150mg/kg) treated group showing less disarrangement of hepatocytes as well.

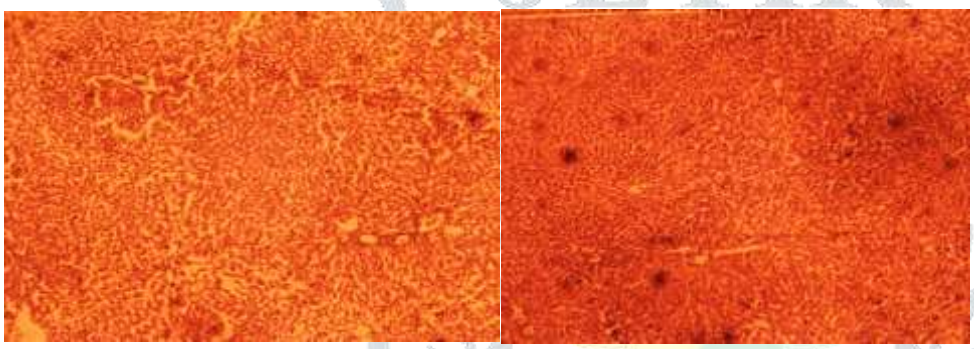

FIG4: - Methanolic medium dose (300mg/kg) showing normal architecture with moderate hepatocyte regeneration activity.

FIG5: - Methanolic high dose (450mg/kg) showing normal architecture with hepatocytes regeneration activity.

6. DISCUSSION

Liver injury caused by toxic chemicals and certain drugs have been recognized as toxicological problem. Hepatotoxicity is one of the very common ailment resulting into serious metabolic disability and even mortality(Patel RK*et al.*, 2008).

Paracetamol is well known drug used as analgesic and antipyretic. Drug is proved to be safe when consumed in a prescribed dose but overdose causes hepatotoxicity due to formation of a toxic metabolite N-acetyl-p-benzoquinone imine (NAPIQI) is formed by cytochrome P450 enzymes in the liver(Manokaran S*et al.*, 2008).

During the study elevated level of entire markers is observed in the group of rats treated with Paracetamol which corresponds to the extensive liver damage. In case of group of rats treated with Silymarin and Methanolic extract of *Holoptelea integrifolia* shows significant decrease in the level of markers.

Herbal drugs having hepatoprotective activity is majorly due the presence of different chemical constituent like flavonoids, phenols, essential oil, lignans etc. Phytochemical evaluation also shows the presence flavonoids in methanolic extract of *Holoptelea integrifolia* which may be responsible for the significant reduction in level of ALT, AST, ALP, total bilirubin and protein.

Also, the literature survey reports the presence of antioxidant activity against 1,1 di-phenyl-2-picrylhydrazyl(DPHH) free radical scavenging activity(Sandhar HK *et al.,* 2011).

Hence the hepatoprotective action of *Holoptelea integrifolia* against paracetamol induced liver toxicity shown by reduction in marker enzymes shows the hepatocytes regeneration activity. These findings during the study have supported the result of the study.

7. CONCLUSION

The present study showed that methanolic extract of *Holoptelea integrifolia* exhibits hepatoprotective action against paracetamol liver toxicity. Hepatoprotective activity was carried out on male wistar albino rats. The level of dose given was 150 mg/kg, 300mg/kg and 450mg/kg body weight out of which the dose level 450mg/kg has shown the significant hepatoprotective action than 150mg/kg and 300mg/kg body weight

8. ACKNOWLEDGEMENT

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