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Hepatoprotective Activity of *Millingtonia Hortensis* in CCL₄ Induced Hepatotoxic Rats

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ABSTRACT

Plants play a great role in the human life and also with other animals. Humans are facing so many health problems day to day because of their regular habits and habitats. In coping that, medicinal plants involvement is very huge. The present approach is also helpful in treating the hepatic problems. This experiment was conducted for seven days to evaluate the hepatoprotective activity of *Millingtonia hortensis lin.* in CCL₄ induced hepato toxic rats. The leaf extracts of these plants were prepared by the maceration technique by aqueous, methanol solvent. The rats were divided in 5 different groups. Among them control, CCL₄ induced (1 ml/kg), CCL₄ + Liver tonic, CCL₄ + MHAE (150 mg/kg) and CCL₄ + MHME (150 mg/kg). After 7 days, on 8th day blood was collected from all group of rats by retro orbital puncture to perform serum tests like SGOT, SGPT and albumin, bilirubin, ALP, Total protein. The reduced values of serum parameters like SGOT, SGPT, and bilirubin total protein in the extract administered rats indicate the hepatoprotective activity of the plant. The aqueous extract of the plant is more effective than to methanol extract.

Key words: Hepatoprotective activity, SGOT, SGPT, ALP, CCL₄

Human an efficient individual on the earth, who can able to utilize all the resources. But the high involvement of human misusing the resources with that he is meeting to negative effects. Because of his regular work stress, he is unable to concentrate on his healthy food or diet. He depends up on the junk food. He also addicts to the other bad habits like consuming alcohol, smoking and drugs. These actions not only harm him but also affect the surrounding persons and resources.

Among the above condition the regular intake of alcohol may cause cirrhosis by which the liver is completely damaged. The liver is a vital organ, which involves in the various metabolic reactions. Damaging the liver causes other disturbances to the body. Though there are allopathic medications are available, they unable to meet the needy person and mostly they may cause side effects. From ancient Indian times the medicinal values of plants are helpful to treat many ailments [1]. There are several plants that are explored with their effective medicinal value. Most of them are unveiled for having the hepatoprotective activity. Plants like *Agrimonia eupatoria*, *Allium sativum*, *Cassia occidentalis*, *Citrullus colocynthis*, *Euphorbia hirta* etc are

already proved by their effective hepatoprotective activity [2]. The present approach is made for knowing and unveiling the importance of plants for their hepatoprotective activity.

MATERIALS AND METHODS

Collection of plant material

The medicinal plant *Millingtonia hortensis lin.* (In Telugu name was Boddumalli) was collected from the village area of Gudur, Mahabubabad District, Telangana State. It was observed and recognized by Prof. V.S. Raju (Retd.), Department of Botany, Kakatiya University, Warangal. The plant was stored in the herbarium of the lab by allocating voucher number (RPU/ZOO/MH/2015).

Extraction procedure

The leaves of collected plant were dried in shade for about 15 days. The leaves were powdered with electrical grinder. The collected coarse powder then passed through No.20 mesh and the fine powder was used for the extraction.

Maceration technique was employed to prepare the extract from leaf powder of the plant. Solvents like methanol and aqueous were used to get the extract. 50g of powder was taken in Stoppard conical flasks; it was mixed with 250ml of solvent and allowed for 24hrs by shaking randomly at room temperature. This 1st filtrate was collected and the remaining marc dissolved in 250ml of solvent with

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vigorous shaking and left for 24 hrs and collected the 2nd filtrate. Then the two filtrates were distilled for collecting extracts and were preserved in well closed amber glass containers at refrigerator temperature.

Model of the animal for experiment

Wistar strain rats about 200gr weighing were brought from the Mahaveer enterprises, Hyderabad. These rats were allowed to habituate to the required laboratory condition (25c, 60% humidity) as per the Institutional Animal Ethics committee protocol (IAEC/03/UPSC/KU/10). Rats fed with appropriate diet (Hypo feed for animals pure) and water ad libium.

Studying the toxicity of extract

Different doses of extracts (150, 200, 250, 300 mg/kg) were given to the animals (rats) (4 groups-6 animals in each group) and they were being observed for seven days. It is noticed that the toxicity of the extract not seen in rats up

to 300 mg/kg. So, for the treatment of the extract the 150 mg/kg dose was selected [3].

Experiment design

Animals were divided into 5 groups of 6 in each
 Group- 1: Control- Treated with Distil water
 Group- 2: CCl₄ + Induced
 Group- 3: CCl₄ + Liv 52
 Group- 4: CCl₄ + MHAЕ 150 mg/kg for 7 days and 5th CCl₄
 Group- 5: CCl₄ + MHME 150 mg/kg for 7 days and 5th CCl₄ [4]

On the next day (8th day), blood was collected from all rats through retro orbital puncture then they were sacrificed. By the centrifugation process the serum samples were separated and used to know SGOT, SGPT, total protein, ALP and bilirubin levels in experimental rats by different tests (through commercially available kits) The results were assessed by one way ANOVA and by Dunnet multiple comparison test with the p<0.05 significance.

Table 1 Body and liver weights

Group	Name	Body weight (g)		Liver weight (g)
		Initial	Final	
I	Control	211.19 ± 8.55	230.65 ± 6.76	8.011 ± 0.60
II	CCl ₄ Induced	224.5 ± 8.04**	210.63 ± 8.75**	7.088 ± 0.26**
III	CCl ₄ + LIV-52	218.11 ± 8.79 ^{ns}	234.33 ± 3.46 ^{ns}	8.19 ± 0.46 ^{ns}
IV	CCl ₄ + MHAЕ150 mg/kg	214.3 ± 5.97 ^{ns}	229.38 ± 6.14 ^{ns}	6.08 ± 0.45**
V	CCl ₄ + MHME150 mg/kg	212.30 ± 4.95 ^{ns}	234.35 ± 4.15 ^{ns}	6.83 ± 0.29**

Table 2 SGOT, SGPT and ALP activity (Units were expressed in international units /litre)

Group	Name	SGOT (U/L)	SGPT (U/L)	ALP (IU/L)
I	Control	41.36 ± 2.39	36.42 ± 1.63	181.20 ± 10.28
II	CCl ₄ Induced	153.04 ± 4.510**	102.95 ± 4.55**	287.78 ± 14.18**
III	CCl ₄ + LIV-52	64.028 ± 2.522**	53.23 ± 3.66**	196.32 ± 5.49*
IV	CCl ₄ + MHAЕ150 mg/kg	95.253 ± 2.939**	64.86 ± 2.91**	249.87 ± 11.97**
V	CCl ₄ + MHME150 mg/kg	83.99 ± 4.05**	59.96 ± 3.21**	231.02 ± 9.99**

Table 3 Total proteins, total bilirubin and albumin

Group	Name	Total Protein (mg/dl)	Total Bilirubin (mg/dl)	Albumin (g/dl)
I	Control	5.77 ± 0.17	0.72 ± 0.20	3.94 ± 0.25
II	CCl ₄ Induced	4.35 ± 0.38**	1.89 ± 0.35**	1.57 ± 0.22**
III	CCl ₄ + LIV-52	6.19 ± 0.33 ^{ns}	0.89 ± 0.54 ^{ns}	2.75 ± 0.26**
IV	CCl ₄ + MHAЕ150 mg/kg	3.99 ± 0.29**	1.59 ± 0.29**	1.91 ± 0.27**
V	CCl ₄ + MHME150 mg/kg	4.03 ± 0.58**	0.97 ± 0.08 ^{ns}	2.26 ± 0.23**

All values are expressed in mean ± SD; n=6 **= p < 0.01, *=p<0.05 compare to control group

ns = p>0.05 not significant compare to control

MHAЕ- *Millingtonia horte nsis* Aqueous Extract

MHME- *Millingtonia hortensis* Methanol Extract

RESULTS AND DISCUSSION

The results are observed that the reduced final body weight and the liver weights of the CCl₄ induced rats. The increased values of the SGOT, SGPT, ALP and total bilirubin are noticed in the CCl₄ induced rats. These values are observed to be decreased in the extracts treated groups. Total protein and albumin values are also decreased in the group -2 CCL induced rats. These values are restored in the LIV-52 given rats. The extracts administered rats are also shown the marginal results with the LIV-52 given rats. The MHME administered rats showed effective activity of the plant extract against the CCL4 toxicity (Table 1-3).

CCl₃* free radicals damage the liver's hepatocytes that are come from the CCl₄. Usually, these free radicals damage the cellular membrane by lipid peroxidation [5]. This reaction causes the release of vital enzymes into the circulation like Serum Glutamate Oxaloacitate Transferase (SGOT), Serum Glutamate Pyruvate Transferase (SGPT), Alkaline Phophatase (ALP) and Bilirubin [6]. Their percentage levels are increased in the CCl₄ given rats. These results are observed to come nearly get down in the MHME and MHAЕ administered rats. The reduction of these values may be because of the decreased toxicity of the CCl₄. The total protein and albumin levels are restored in the extract treated rats. This might be the regeneration capacity and

healing activity of the extract to the liver. The presence of the flavonoids, tannins, Saponins help in fighting against the free radicals [7-8]. The present plant extracts also proved that the flavonoids, phenols, saponins and tannins in the phytochemical analysis. These might have play in combating the free radicals of CCl₄ and causing hepatoprotective activity. Glutathione levels may be increased in the extracts administered rats with which the counter action of free radicals may be occurred [9]. Similar results are observed in the experiments of *Zanthoxylum*

armatum administered paracetamol induced toxicity rats [10].

CONCLUSION

The decreased values of serum parameters like SGOT, SGPT, and bilirubin, total protein in the extract administered rats indicate the hepatoprotective activity of the plant. The aqueous extract of the plant is more effective than to methanol extract.

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