

## PROTECTIVE EFFECT OF EXTRACT OF CURCUMA CAESIA IN ETHANOL INDUCED HEPATIC DAMAGE IN RATS

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### Abstract

The objective of present research work is to evaluate protective effect of extract of curcuma caesia in ethanol induced hepatic damage in rats. This study was to compare the hepatoprotective activity of aqueous extract of *C. caesia* and standard drug silymarin. The LD50 of *C. caesia* was found to be safe up to 2000 mg/kg. Thus, it would be safe to use this extract as a hepatoprotective agent. The result shows significant increase in levels of serum SGPT, SGOT and ALP confirmed the hepatotoxicity in the group of rats administered with ethanol as shown in table 1. Pretreatment group of rats with aqueous extract of *C. caesia* at dose level of 200 mg/kg showed significant ( $P < 0.001$ ) different from control group, which proves at this doses the extract has hepatoprotective activity but not sufficient activity by restoring at the levels of SGPT, SGOT and ALP respectively. Groups of rats treated with aqueous extract of *C. caesia* at dose level of 400 mg/kg showed more significant ( $P < 0.001$ ) different from ethanol control group proved by improvement in levels of the SGPT, SGOT and ALP respectively. The animals treated with the silymarin (200 mg/kg) showed slightly higher significant ( $P < 0.001$ ) reduction in rise in the serum enzymes level in comparison to ethanol control group. The aqueous extract of *C. caesia* used in the study preserved the structural integrity of the hepatocellular membrane in a dose dependent manner as evident from the protection provided as compared to the enzyme levels in ethanol control group rats. The aqueous extract of *C. caesia* at dose level 400 mg/kg showed prominent hepatoprotection in comparison to the ethanol control group and silymarin pretreated group rats.

**Keyword :** Hepatoprotective, toxicity, diagnosis, Medicine, Percentage yield.

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**Introduction :** Man's existence on this earth has been made possible only because of the vital role played by plant kingdom. Medicinal plants existing even before human being made their appearance on the earth. Traditional medicine using herbal drugs exists in every part of the world. The major areas are Chinese, Indian and European traditions. The philosophies of these traditional medicines have some resemblance to each other but differ widely from modern Western medicine. In view of the progress of Western medicine not only new synthetic drugs but also herbal drugs have to fulfill the international requirements on quality, safety and efficacy. Herbal drugs have the advantage of being available for patients in the geographical area of the special traditional medicine. Practically every country develops its own medical system, which includes the ancient civilization of China, Egypt and India. Thus, the Indian Medical System-Ayurveda came into existence. The raw materials for Ayurvedic medicines were mostly obtained from plant sources in the form of crude drugs such as dried herbal powders or their extracts or mixture of products. Also, Siddha, Unani and Tibb are traditional health care systems have been flourishing for many centuries. Apart from these systems there has been a rich heritage of ethnobotanical usage of herbs by various colorful tribal communities in the country. Medicinal plants contain inherent active ingredients to cure disease or relieve pain. The use of traditional medicines and medicinal plant in most developing countries as therapeutic agent for the maintenance of good health has been widely observed. The world health organization estimated that 80% of the population of developing countries relies on traditional medicines, mostly herbal plant drugs for their primary health care. The medicinal property of plant could be based on the anti-oxidant, antimicrobial, antipyretic effect of the phytochemicals present. Traditionally, herbs have been considered to be non-toxic and have been used for treating various problems by the general public and/or traditional medicine doctors worldwide. Although, the literature has documented several toxicity resulted from the use of herbs on many occasions, still the potential toxicity of herbs has not been recognized by the general public or by professional groups of traditional medicine. The use of medicinal plants as raw materials in the production of drug is gaining popularity.

India perhaps the largest producer of medicinal herbs and is called Botanical Garden of the World. Medicinal herbs have been in use for thousands of years, in one form or another, under the indigenous systems of medicine like Ayurveda, Sidha and Unani. On earth, around 3.6 lakh species of medicinal plants are present, among these 1.4 lakh species are in India. A latest survey indicates that about 70000 plants are used in traditional systems of medicines.<sup>3</sup> All over the world, plants were used as main source of medicines by ancestors. The rise of modern western medicine was initially accompanied by a decline in the practice of herbalism in all cultures and it was believed that synthetic chemicals were best medicines to treat illness and cure disease<sup>1-5</sup>

## Materials and Methods<sup>6-23</sup>

### MATERIALS AND METHODS:

**Plant material:** The plant material of *Curcuma caesia* was purchased from local market in the form of dried rhizomes. Further identification has also been done from Safia College, Bhopal (M.P.)

**Preparation of extract:** After drying at 37 degree Celsius for 24 hours the plant material was ground into powder. Exposure to sunlight was avoided to prevent the loss of active components. One liter of double distilled water was mixed with 200 g of powdered *Curcuma longa* rhizomes, filtered with filter paper and the extracted liquid was subjected to water bath evaporation to remove the water. For water bath evaporation, liquid extract material was then placed into a beaker and subjected to water bath evaporation at 70 degree Celsius temperature for 7 to 10 hours daily for 2 to 3 days until a semisolid state of extracted liquid is obtained. The semisolid extract produced was kept in the deep freezer at -20 degree Celsius overnight and then subjected to freeze drying. Extract obtained by this method was then weighed and stored at 22 degree Celsius in desiccators until further use.

**Calculation of percentage yield:** The percentage yield of yield of each extract was calculated by using formula: Percentage yield = Weight of extract x 100/ Weight of powdered drug taken

**Chemicals:** All reagents used in the study were of high purity. All chemicals such as ethanol, formalin, xylene and DMSO were purchased from Sigma Aldrich Chemical (Malaysia). Silymarin purchased from Sigma Aldrich Chemical (China) were also used in the experiments.

### Experimental animals:

Experiments were carried out on healthy adult male albino wistar rats weighing 180±20 grams. They were raised in

the animal house. Animals were housed in polypropylene cages with stainless steel grill top at 25 ± 2oC.

### Result and Discussion –

The aqueous extract of *Curcuma caesia* were evaluated for its hepatoprotective activity. The various observation and results obtained from evaluation are discusses in this chapter.

### Physical characteristics of extracts:

Finally extraction was done with water and % yield was found to be 15.66 % w/w and their characteristics are reported in table- 7.1

**Table- 7.1 Physical characteristics of extracts**

Extracts	Consistency	Colour	Odour	Taste	% Yield
Aqueous	Semi- solid	Dark Brown	pungent	bitter	15.66

**Acute toxicity studies:** All the rats that received aqueous extract of *Curcuma caesia* either at high dose up to 2000 mg/kg or low dose were found to be safe. No mortality or toxic symptoms were observed during the entire duration of the study. Aqueous extract of *Curcuma caesia* showed a stable compliance towards the rats and proved to be safe.

**Effect of aqueous extract of *Curcuma caesia* on Organ to Body Weight Indices (OBWI) in ethanol induced hepatotoxicity in albino wistar rats:** Ethanol has enhanced the OBWI. This clearly indicates that there is a significant hepatic damage due to ethanol. Treatment with silymarin, 200mg/kg and 400mg/kg of

*C. caesia* rhizomes (aqueous extract) has significantly (P<0.05) brought down the elevated OBWI in experimental animals.

Figure 7.1: Effect of aqueous extract of CC on liver organ body weight indices(OBWI)

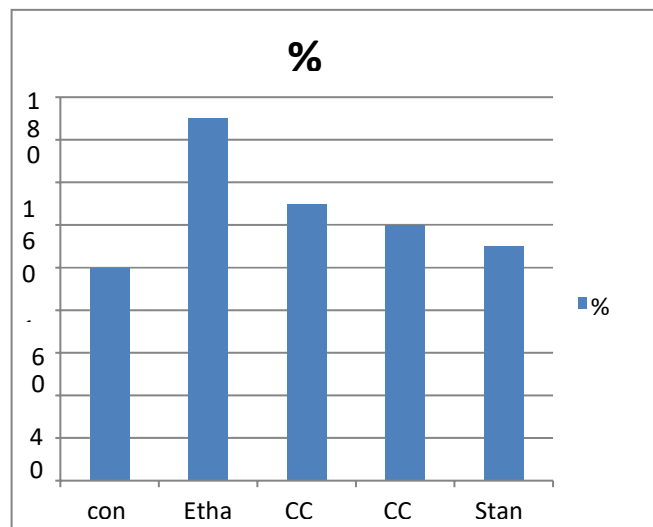


Table 7.2 shows the effect of *C. caesia* on liver enzymes % SGPT, % SGOT and % ALP level in serum of ethanol induced hepatotoxicity in male albino wistar rats. The control had shown the low level of liver enzymes in serum but after ethanol treatment, enzymes level increased. Whereas after administration of *C. caesia* at the doses of 200 mg/kg, and 400 mg/kg bw po in ethanol intoxicated rats, the enzymes level significantly reduced.

**Table: 7.2 Effect of aqueous extract of *Curcuma caesia* on liver enzymes in ethanol induced hepatotoxicity in albino wistar rats**

Treatment	Dose	SGPT (%)	SGOT (%)	ALP (%)
Control	Normal	100.0	100.0	100.0 ±
	Saline10 ml/kg bw po	±6.81	±12.65	17.41
Ethanol	10 ml/kg bw po	340.0 ± 18.40	315.0 ± 18.20	305.0 ± 17.11
CC Extract	200 mg/kg bwpo	230.0 ± 11.70 ***	200.0 ± 10.11* **	234.0 ± 12.85* **
CC Extract	400 mg/kg bwpo	200.0 ± 11.18 ***	180.0 ± 10.12 ***	215.0 ± 11.60 ***
Silymarin	200 mg/kg bwpo	130.0 ± 6.60* **	120.0 ± 6.65** *	140.0 ± 15.52 ***

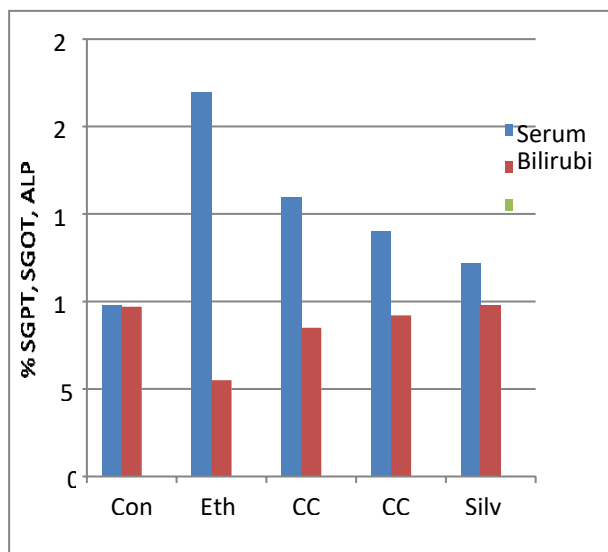


Figure 7.2: Effect of aqueous extract of CC on liver enzymes SGPT,SGOT & ALP

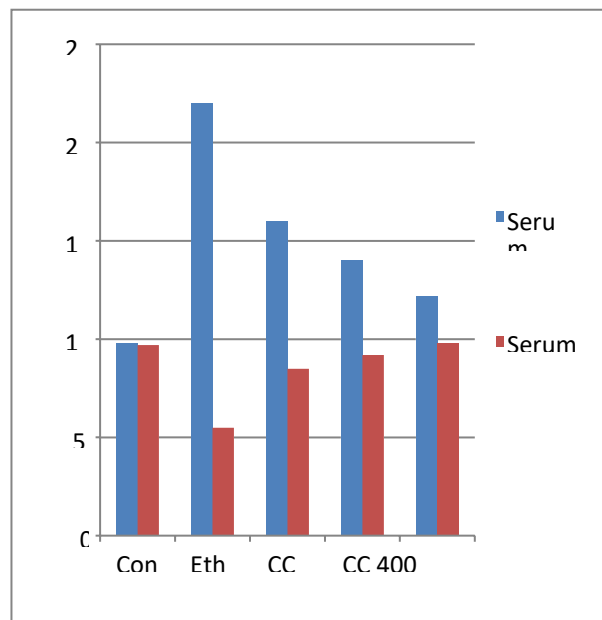
\*P<0.05, compared to control group (One way ANOVA followed by Dunnett's t test).

#P<0.05, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

Table 7.3 shows the effect of C. caesia on % bilirubin and protein level in serum of ethanol induced hepatotoxicity in male albino wistar rats. The control had shown the low level of bilirubin in serum but after ethanol treatment, bilirubin level increased. Whereas after administration of C. caesia at the doses of 200 mg/kg, and 400 mg/kg bw po in ethanol intoxicated rats, the bilirubin level significantly reduced. Control shown high level of serum protein but after ethanol treatment serum protein level reduced. Whereas after administration of doses of C. caesia extract, the serum protein level increased significantly.

Table: 7.3 Effect of aqueous extract of Curcuma caesia on % serum bilirubin and protein level in ethanol induced hepatotoxicity in albino wistar rats

Treatment	Dose	Serum Bilirubin (%)	Serum Protein (%)
Control	Normal		
	Saline 10 ml/kg bw po	98.0 ± 5.51	97.0 ± 5.50
Ethanol	10 ml/kg bwpo	220.0 ± 12.50	55.0 ± 4.43
CC Extract	200 mg/kg bwpo	160.0 ± 10.11 <sup>***</sup>	85.0 ± 5.44 <sup>***</sup>
CC Extract	400 mg/kg bwpo	140.0 ± 8.50 <sup>***</sup>	92.0 ± 6.00 <sup>***</sup>
Silymarin	200 mg/kg bwpo	122.0 ± 6.66 <sup>***</sup>	98.0 ± 5.20 <sup>***</sup>



**Figure 7.3: Effect of aqueous extract of CC on Serum bilirubin and protein.**

\* $P < 0.05$ , compared to control group (One way ANOVA followed by Dunnett's *t* test).

# $P < 0.05$ , compared to Positive control group (One way ANOVA followed by Dunnett's *t* test).

### Results of Histopathological studies

Histopathological profile of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Figure A). Group II animals exhibited disarrangement of normal hepatic cells with intense centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration (Figure B). The liver sections of the rats treated with aqueous extract of *C. caesia* followed by ethanol intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles (Figure D and E). The liver sections of the rats treated with silymarin followed by ethanol intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles (Figure C).

**DISCUSSION:** This study was undertaken to evaluate the hepatoprotective activity of aqueous extract of *C. caesia* on ethanol induced hepatotoxic in male albino wistar rats. This study was to compare the hepatoprotective activity of aqueous extract of *C. caesia* and standard drug silymarin. The LD<sub>50</sub> of *C. caesia* was found to be safe up to 2000 mg/kg. Thus, it would be safe to use this extract as a

hepatoprotective agent. The result shows significant increase in levels of serum SGPT, SGOT and ALP confirmed the hepatotoxicity in the group of rats administered with ethanol as shown in table 1. Pretreatment group of rats with aqueous extract of *C. caesia* at dose level of 200 mg/kg showed significant ( $P < 0.001$ ) different from control group, which proves at this doses the extract has hepatoprotective activity but not sufficient activity by restoring at the levels of SGPT, SGOT and ALP respectively. Groups of rats treated with aqueous extract of *C. caesia* at dose level of 400 mg/kg showed more significant ( $P < 0.001$ ) different from ethanol control group proved by improvement in levels of the SGPT, SGOT and ALP respectively. The animals treated with the silymarin (200 mg/kg) showed slightly higher significant ( $P < 0.001$ ) reduction in rise in the serum enzymes level in comparison to ethanol control group. The aqueous extract of *C. caesia* used in the study preserved the structural integrity of the hepatocellular membrane in a dose dependent manner as evident from the protection provided as compared to the enzyme levels in ethanol control group rats. The aqueous extract of *C. caesia* at dose level 400 mg/kg showed prominent hepatoprotection in comparison to the ethanol control group and silymarin pretreated group rats.

### CONCLUSION:

In this study, hepatoprotective activity of the aqueous extract of *C. caesia* was studied. The aqueous extract of *C. caesia* at the dose of 400 mg/kg showed very prominent and similar to silymarin hepatoprotective activity as demonstrated by significant ( $P < 0.001$ ) decrease in enzyme levels and preserved the structural integrity of the hepatocellular membrane as evident from the protection provided as compared to the ethanol control group rats. Identification of natural compound of plant will help to develop new therapeutically agents. The results obtained from present study shows that this plant is a good natural source for hepatoprotective activity. As this plant is easily available and the aqueous extract is showing better activity, this suggests that this plant is a cost effective natural treatment available in market. Further clinical trials should be done in order to develop a prominent formulation that will be useful for public. As the cost of the treatment is rising, developing a cost effective remedies will definitely give a better option and opportunities to treat chronic diseases.

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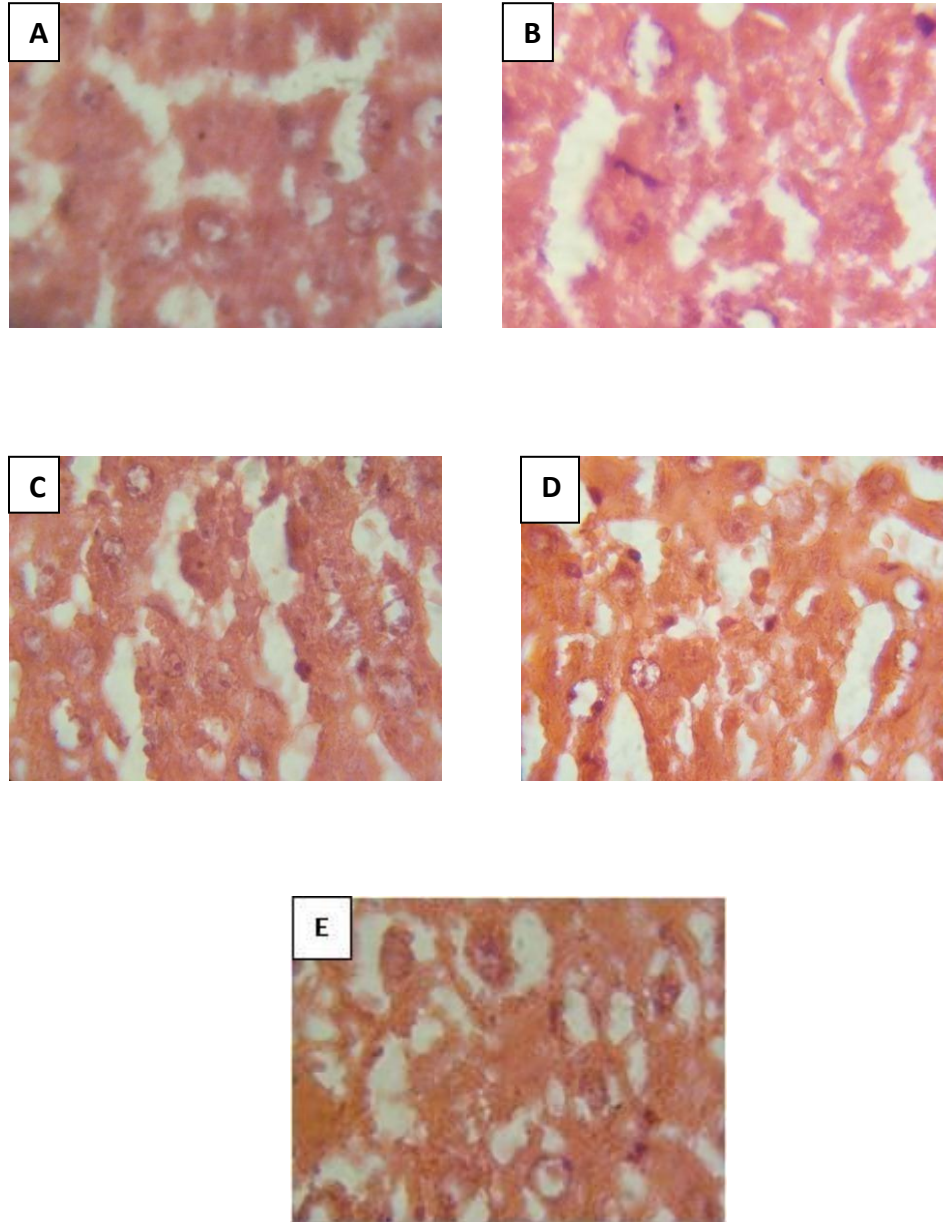


Figure 7.4 Light microscopic analysis of rat liver sections of normal rats and treated with drug administration. (A) Normal control; (B) Ethanol; (C) Silymarin; (D) CC-200; (E) CC-400