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HEPATOPROTECTIVE ACTIVITY OF *PLUMERIA OBTUSA* LEAVES EXTRACT AGAINST CCL_4 AND ETHANOL INDUCED HEPATIC DAMAGE IN RATS

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ABSTRACT

Liver, the key organ of metabolism and excretion, is constantly endowed with the task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. The aim of the present study was to evaluate hepatoprotective activity of aqueous and ethanolic extracts of *Plumeria obtusa* in wistar rats. It was found that *Plumeria obtusa* aqueous and ethanolic extracts possess significant hepatoprotective activity.

Keywords: Hepatoprotective activity, *Plumeria obtusa,* Xenobiotics, Liver

INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role 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 **[1]**. Stomach related hyperacidity and ulcer are very common causing human suffering today. It is an imbalance between damaging factors within the lumen and protective mechanisms within the gastro duodenal mucosa **[2]**. Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model. Carbon

tetrachloride (CCl_4) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts [**3, 4**].

Plumeria obtusa is native to the Greater Antilles, Florida, northern Central America and southern Mexico. Cultivation is common in heater parts of the world, including Southeast Asia **[5]**. In the Caribbean Islands, people use the bark as a diuretic. They also use the latex to stimulate purging. In many islands in the Pacific, women can tactfully affirm their marital status by inserting a Plumeria flower on the right ear if they are single or on the left if they are married. In Hawaii, the flowers are very trendy and often located in floral leis. Thus the objective of the present study was designed to test the hepatoprotective activity of the ethanol and aqueous extracts of plant material of above specified plants against carbon tetrachloride and ethanol induced liver damage in rats.

MATERIALS AND METHODS

PLANT MATERIALS AND PREPARATION OF EXTRACTS

The leaves of *Plumeria obtusa* were gathered in the month of July 2016 from local areas of Indore (M.P.) and were identified and authenticated by Dr. S.N. Dwivedi, Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, M.P. what's more, kept in our Laboratory, Voucher specimen No. J/BOT/H- 139. The leaves of *Plumeria obtusa* were dried under shade and then powdered with a mechanical grinder. The powders were passed through sieve No. 40 and stored in an airtight container for further uses.

The dried powder of leaves of *Plumeria obtusa* were separated with Ethanol (95%) in a soxhlet apparatus. Aqueous extract was set up by cold maceration process by utilizing separate amount of powder. The solvents were evacuated by refining under diminished weight and the subsequent semisolid mass was vacuum dried utilizing rotary flash evaporator.

DRUGS AND CHEMICALS

CCl_4 was purchased from the local market of Indore (M.P.). Ethanol is also purchased from the Dawa Bazar, Indore (M.P.).

ANIMALS

Swiss albino rats (100-120 g) and mice of either sex and of approximate same age, used for this study, were procured from listed supplier Palika Plaza Market, Nagar Nigam, Indore (M.P.) India. They were housed in well ventilated stainless-steel cages at room temperature (24±2ºC) in hygienic condition under natural light and dark schedule. The animals were fed with standard pellet diet and water ad libitum.

ACUTE TOXICITY STUDY

The extracts of *Plumeria obtusa* were tested for their toxicity as per Organization for Economic Co-operation and Development test guidelines (OECD)-No. 423 for acute toxic classic method **[6]**. Swiss albino of single sex (male; n=6) were grouped into three. After one week of maintenance at laboratory conditions, each group of animals were administered orally 2000 mg/kg of a test extract and the animals were kept under observation to monitor mortality, physiological and psychological condition such as skin changes, morbidity, aggressiveness, oral secretions, sensitivity to sound and pain and respiratory movements.

EXPERIMENTAL DESIGN FOR HEPATOPROTECTIVE ACTIVITY

Albino rats (100-120 gms) used in the present studies were procured from listed suppliers of Indore (M. P.), India. The animals were fed with standard pellet diet and water *ad libitum*. All the animals were acclimatized for a week before use.

CCL_4 INDUCED MODEL

The rats were divided into 5 groups of 3 animals in each.

Group I : Received vehicle Gum acacia (5mg/kg p.o.) for 7 days, and served as normal control.

Group II : Received vehicle Gum acacia (5 mg/kg p.o.) for 7 days once daily. Carbon tetrachloride 1ml/kg in 50% v/v olive oil on 7^{th} day.

Group III : Received standard drug Silymarin (25 mg/kg) for 7 days once daily, CCl_4 1ml/kg in 50% v/v olive oil on 7^{th} day.

Group IV : Received aqueous extract of leaves of *Plumeria obtusa* (500mg/kg) for 7 days once daily, CCl_4 1ml/kg in 50% v/v olive oil on 7^{th}day.

Group V : Received ethanolic extract of leaves of *Plumeria obtusa* (500mg/kg) for 7 days once daily, CCl_4 1ml/kg in 50% v/v olive oil on 7^{th}day.

On the 7^{th} day food and water were withdrawn after giving the last doses of aqueous and ethanolic extracts. After 36 hours the blood samples were collected and allowed to clot and serum was separated by centrifuge at 2500 rpm for 15 min and analyzed for various biochemical parameters.

ASSESSMENT OF LIVER FUNCTION

The liver was removed and weighed. Biochemical parameters i.e., Serum glutamic Pyruvate transaminase (SGPT), serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline phosphatase (ALP) were analyzed according to the reported methods.

HISTOPATHOLOGICAL STUDIES

Liver slices fixed for 12 hrs in Bouin's solution were processed for paraffin embedding following standard micro technique. 5µ section of the livers stained with alum haematoxylin and eosin, were observed microscopically for histopathological changes i.e., normal liver, damaged and recovered liver were studied and compared.

ETHANOL INDUCED MODEL

The rats were divided into 5 groups of 3 animals in each.

Group I : Received vehicle gum acacia (5mg/kg. p.o.) for 5 days, and served as normal control.

Group II : Received vehicle gum acacia (5 mg/kg p.o.) for 5 days once daily 50% Ethanol 5ml/kg on 5th day, and served as disease control.

Group III : Received Silymarin (25 mg/kg) for 5 days once daily, 50% Ethanol 5ml/kg on 5th day.

Group IV : Received aqueous extract of leaves of *Plumeria obtusa* (500mg/kg) for 5 days once daily and 50% Ethanol 5ml/kg on 5th day.

Group V : Received ethanolic extract of leaves of *Plumeria obtusa* (500mg/kg) for 5 days once daily and 50% Ethanol 5ml/kg on 5th day.

On the 5th day food and water were withdrawn after giving the last dose of aqueous and ethanolic extracts. After 24 hours the blood samples were collected and allowed to clot and serum was separated by centrifuge at 2500 rpm for 15 min and analyzed for various biochemical parameters.

ASSESSMENT OF LIVER FUNCTION

The liver was removed and weighed. Biochemical parameters i.e., Serum glutamic Pyruvate transaminase (SGPT), serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline phosphatase (ALP) were analyzed according to the reported methods.

HISTOPATHOLOGICAL STUDIES

Liver slices fixed for 12 hrs in Bouin's solution were processed for paraffin embedding following standard micro technique. 5µ section of the livers stained with alum haematoxylin and eosin, were observed microscopically for histopathological changes i.e., normal liver, damaged and recovered liver were studied and compared [**7-12]**.

RESULTS AND DISCUSSION

HEPATOPROTECTIVE STUDIES (CCL_4 INDUCED MODEL)

Liver plays a key role in regulation of physiological processes. it is involved in several functions such as metabolism, secretion and storage. Furthermore

detoxification of a variety of drugs and xenobiotics occurs in liver. The bile secreted by the liver has, among other things, an important role in digestion. Liver diseases are the most serious ailments.

The results of biochemical parameters revealed that the elevation of enzyme level in CCl_4 treated group, are almost restored to the normal level in the extract treated group.

EFFECT ON SGPT

Ethanolic extract and aqueous extract of leaves of *Plumeria obtusa* showed significant hepatoprotective activity as they reduced SGPT to 94.84 ± 4.62 and 92.24 ± 8.24 as compared to the hepatotoxic control 154.8 and hence the extracts of leaves of *Plumeria obtusa* showed significant hepatoprotective activity. The results of treatment with extract of *Plumeria obtusa* are tabulated in **Table 1**.

SGPT is a cytosolic enzyme primarily present in the liver. The level of SGPT in serum increase due to leakage of this cellular enzyme into plasma by CCl_4 induced hepatic injury. Serum level of SGPT can increase due to damage of the tissue producing acute hepatic necrosis. Since the extract of *Plumeria obtusa* significantly reduced the level of SGPT, this suggests that the extracts possess significant hepatoprotective activity.

EFFECT ON SGOT

Ethanolic and aqueous extract of leaves of *Plumeria obtusa* showed significant hepatoprotective activity as they reduced SGOT to 208.48 ± 8.64 and 204.24± 9.64 as compared to the hepatotoxic control 348.24± 10.42 and hence the extracts showed significant hepatoprotective activity. The results of treatment with extracts of leaves of *Plumeria obtusa* are tabulated in **Table 1**.

SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. Liver toxicity elevated the SGOT level in serum due to the damage to the tissue producing acute necrosis such as several viral hepatitis and acute cholestasis. Since the extract of *Plumeria obtusa* significantly reduced the level of SGOT, this suggests that the extracts possess significant hepatoprotective activity.

EFFECT ON ALP

Ethanolic and aqueous extract leaves of *Plumeria obtusa* showed significant hepatoprotective activity as they reduced ALP to 208.46 ± 9.64 and 202.42 ± 8.48 as compared to the hepatotoxic control 360.20 ± 8.82 and hence the extract of leaves of *Plumeria obtusa* showed significant hepatoprotective activity. The results of treatment with extracts of *Plumeria obtusa* are tabulated in **Table 1**.

In case of toxic liver, alkaline phosphate levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchyma or duct cells. Since the extract of *Plumeria obtusa* significantly reduced the level of ALP, this suggests that the extracts possess significant hepatoprotective activity.

EFFECT ON LIVER WEIGHT

The liver weight of animals treated with Ethanolic and aqueous extracts of the leaves of *Plumeria obtusa* were compared with that of the standard drug Silymarin (25mg/kg) treated ones.

The ethanolic and aqueous extracts of the leaves of *Plumeria obtusa* exhibited significant decrease in the weight of liver as that of the standard drug Silymarin and thus suggest that the both extracts of leaves of *Plumeria obtusa* possess significant hepatoprotective activity **(Table 2)**.

HEPATOPROTECTIVE STUDIES (ETHANOL INDUCED MODEL)

The result of biochemical parameter revealed that the elevation of enzyme level in Ethanol induced treated group almost restored to the normal level in the extracts treated groups.

EFFECT ON SGPT

Ethanolic extract and aqueous extract of leaves of *Plumeria obtusa* showed significant hepatoprotective activity as they reduced SGPT to 78.22 ± 4.82 and 76.20 ± 2.28 as compared to the hepatotoxic control 112.4 ± 4.20 and hence the extracts of *Plumeria obtusa* showed significant hepatoprotective activity. The results of treatment with extracts of *Plumeria obtusa* are tabulated in **Table 3**.

SGPT is a cytosolic enzyme primarily present in the liver. The level of SGPT in serum increase due to leakage of this cellular enzyme into plasma by Ethanol induced hepatic injury. Serum level of SGPT can increase due to damage of the tissue producing acute hepatic necrosis. Since the extract of *Plumeria obtusa* significantly reduced the level of SGPT, this suggests that the extracts possess significant hepatoprotective activity.

EFFECT ON SGOT

Ethanolic extract and aqueous extract of leaves of *Plumeria obtusa* showed significant hepatoprotective activity as they reduced SGOT to 202.24 ± 8.64 and 200.22 ± 4.20 as compared to the hepatotoxic control 298.00 ± 4.60 and hence the extracts of *Plumeria obtusa* showed significant hepatoprotective activity. The results of treatment with extracts of *Plumeria obtusa* are tabulated in **Table 3**.

SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney.

Liver toxicity elevated the SGOT level in serum due to the damage to the tissue producing acute necrosis such as several viral hepatitis and acute cholestasis. Since the extract of *Plumeria obtusa* significantly reduced the level of SGOT, this suggests that the extracts possess significant hepatoprotective activity.

EFFECT ON ALP

Ethanolic and aqueous extract of leaves of *Plumeria obtusa* showed significant hepatoprotective activity as they reduced ALP to 208.00 ± 6.48 and 206.20 ± 4.88 as compared to the hepatotoxic control 318.46 ± 10.82 and hence the extracts of *Plumeria obtusa* showed significant hepatoprotective activity. The results of treatment with extracts of *Plumeria obtusa* are tabulated in **Table 3**.

Table 1: Effect of aqueous and ethanolic extracts of leaves of *Plumeria obtusa* on CCl_4 induced hepatotoxicity in rats

Treatment	Total Bilirubin (mg%)	Direct Bilirubin (mg%)	SGOT (µ/min/l)	SGPT (µ/min/l)	ALP (µ/min/l)
Normal	0.46 ± 0.22	0.42 ± 0.68	184.04 ± 2.4	78.42 ± 2.42	193.0 ± 6.4
Induced (CCl_4 2g/kg)	8.64 ± 2.48	7.48 ± 8.64	348.42± 10.42	154.8± 8.46	360.20±8.82
Standard (Silymarin 25mg/kg)	0.54 ±4.40**	0.50 ±0.22**	198.00±9.48**	89.00±8.82**	200.22 ±10.66**
Aqueous extract (500mg/kg)	0.58 ± .64**	0.52 ±0.26**	204.24± 9.64**	92.24±8.24**	202.42± 8.48**
Ethanolic extract (500mg/kg)	0.64 ± 4.62*	0.54 ± 0.28*	208.48± 8.64*	94.84± 4.62*	208.46± 9.64*

Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * P<0.01, ** P<0.001, when compared with respective control

Table 2: Effect of aqueous and ethanolic extracts of leaves of *Plumeria obtusa* on liver weight variation of CCl_4 induced hepatotoxicity in rats.

Treatment	Liver weight in g/100g
Normal	6.82 ± 0.46
Induced (CCl_4 2g/kg)	8.22 ± 0.24
Standard (Silymarin 25mg/kg)	7.12 ± 0.26**
Aqueous extract (500 mg/kg)	7.20 ± 0.24**
Ethanolic extract (500 mg/kg)	7.24 ± 0.62*

Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * P<0.01, ** P<0.001, when compared with respective control

Table 3: Effect of aqueous and ethanolic extracts of leaves of *Plumeria obtusa* on ethanol induced hepatotoxicity in rats

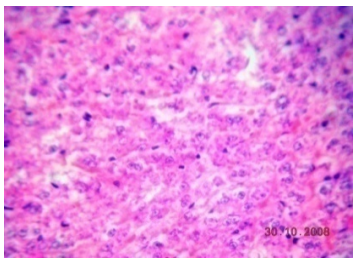
Treatment	Total Bilirubin(mg%)	Direct Bilirubin(mg%)	SGOT (µ/min/l)	SGPT (µ/min/l)	ALP (µ/min/l)
Normal	0.46 ± 0.22	0.42 ± 0.68	184.04 ± 2.4	78.42 ± 2.42	193.0 ± 6.4
Control (Ethanol 1g/kg)	9.82 ± 2.82	6.28 ± 3.36	298.00 ± 4.60	112.4± 4.20	318.46 ±10.82
Standard (Silymarin 25mg/kg)	0.54 ± 0.02**	0.42 ± 2.86**	186.48± 8.52**	68.42±8.46**	196.00 ± 8.24**
Aqueous extract (500mg/kg)	0.58 ± 0.44**	0.48 ± 0.46**	200.22 ± 4.20**	76.20 ± 2.28**	206.20 ± 4.88**
Ethanolic extract (500mg/kg)	0.60± 0.22*	0.54 ± 0.24*	202.24 ± 8.64*	78.22 ± 4.82*	208.00 ± 6.48*

Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * P<0.01, ** P<0.001, when compared with respective control.

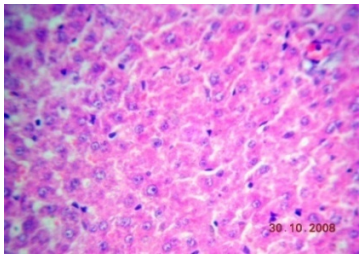
Table 4: Effect of aqueous and ethanolic extracts of leaves of *Plumeria obtusa* on liver weight variation of Ethanol induced hepatotoxicity in rats

Treatment	Liver weight in g/100g
Normal	6.82 ± 0.46
Induced (Ethanol1g/kg)	7.48 ± 0.28
Standard (Silymarin 25mg/kg)	7.12 ± 0.48**
Aqueous extract (500 mg/kg)	7.28 ± 0.22**
Ethanolic extract (500 mg/kg)	7.36 ± 0.68*

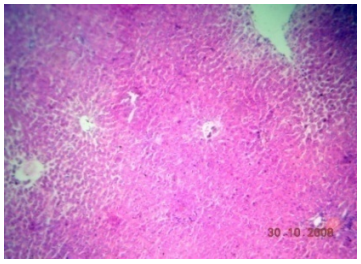
Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * P<0.01, ** P<0.001, when compared with respective control.



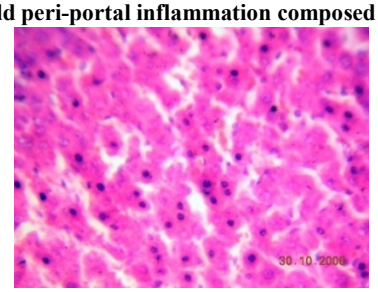
a) Normal: The architecture is normal. The central veins, sinusoids and portal triads appear normal. The hepatocytes show moderate cytoplasm and round to oval nuclei. There is no periportal inflammation



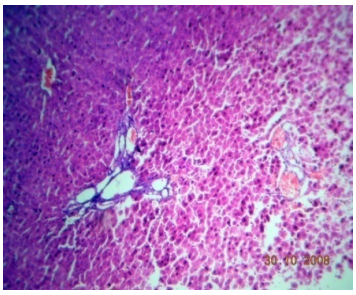
b) CCl_4 induced (500mg/kg): The central veins show dilatation and congestion. The hepatocytes show feathery degeneration. The portal triads show mild peri-portal inflammation composed of lymphocytes



c) Silymarin (25mg/kg): The central veins appear normal. The hepatocytes show feathery degeneration. The portal triads show mild peri-portal inflammation composed of lymphocytes.

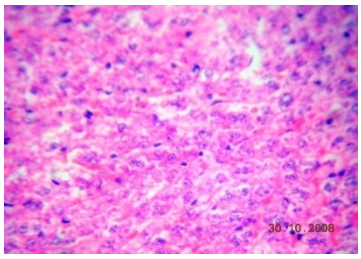


d) Aqueous extract (500 mg/kg): The architecture is normal. The central veins, sinusoids and portal triads appear normal. The hepatocytes show moderate cytoplasm and round to oval nuclei.

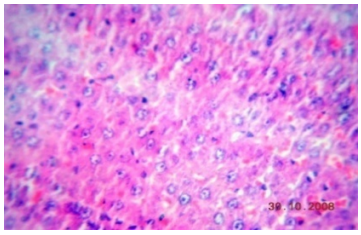


e) Ethanolic extract (500 mg/kg): The hepatocytes show moderate cytoplasm and moderately enlarged pleomorphic and hyperchromatic nuclei. The portal triads show mild peri-portal inflammation composed of lymphocytes. The central veins are normal

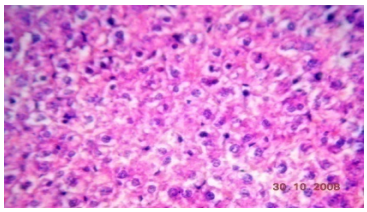
Figure 1: (a-e) Diagram showing histopathologic section of liver of rats in CCl_4 induced hepatotoxicity



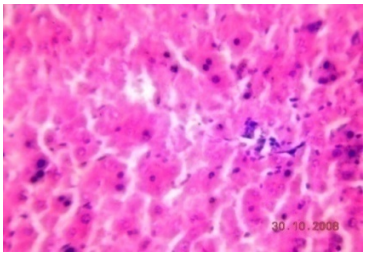
a) Normal: The architecture is normal. The central veins, sinusoids and portal triads appear normal. The hepatocytes show moderate cytoplasm and round to oval nuclei. There is no periportal inflammation



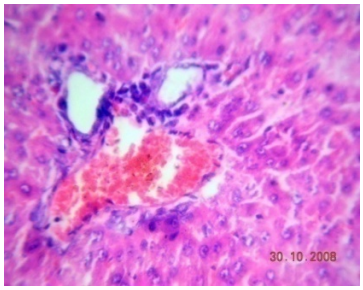
b) Ethanol induced (1g/kg): The central veins show mild dilatation and congestion. The hepatocytes are normal. The portal triads appear normal.



c) Silymarin (25mg/kg): Section shows large dilated and congested central veins. The hepatocytes are normal. The portal triads appear normal.



d) Aqueous extract (500 mg/kg): The architecture shows patchy necrosis. The hepatocytes shows moderate cytoplasm and round nuclei. The portal triads appear normal.



e) Ethanolic extract (500 mg/kg): The architecture is normal. The hepatocytes shows moderate cytoplasm and round nuclei. The portal triads appear normal

Figure 2: (a-e) Diagram showing histopathologic section of liver of rats in Ethanol induced hepatotoxicity

CONCLUSION

The present investigation indicates that, the aqueous and ethanolic extracts of *Plumeria obtusa* leaves, exhibit hepatoprotective effect against CCl_4 & Ethanol induced hepatic damage. Thus this plant should be recorded for its global acceptance as herbal drug.

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