# HEPATOPROTECTIVE ACTIVITY OF TURNERA APHRODISIACA LEAVES AGAINST DICLOFENAC INDUCED LIVER INJURY IN RATS

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**Abstract**

**Introduction:** Traditional medicine is the sum of knowledge, skills, beliefs and experiences used in the maintenance of health and in the diagnosis, prevention, improvement or treatment of physical and mental illness. Drugs can damage the liver cells in their original form or in various intermediate stages.

**Objective:** Effects of Ethanol extract of Turnera aphrodisiaca in treatment of Diclofenac induced hepatotoxicity in lab animals.

**Methods:** Group I was given a 2% w/v acacia solution, Group II was given 150 mg/kg/b.w. of diclofenac, Group III received 150 mg/kg/b.w. of diclofenac and standard silymarin, and Group IV and V received 200 and 400 mg/kg b.w. of EETA. Animals were sacrificed on the 29th day and their livers were separated, cleaned, and preserved in 10% neutral formalin for histopathological research.

**Results:** The serum biochemical indices of the EETA-treated groups at 200 and 400 mg/kg were significantly lower than those of the diclofenac-treated group. White spots, a prominent blood vein, and a normal hepatic lobule structure were all seen in histopathological studies. The Diclofenac-treated group had severely harmed hepatic parenchyma. The hepatic parenchyma recovered, there was minimal congestion, and there were micro vesicular changes in the EETA-treated groups.

**Key words:** EETA, diclofenac, groups, Turnera aphrodisiaca.

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## INTRODUCTION

According to World Health Organization (WHO), 80% of the people living in developing countries rely almost exclusively on traditional medicine for their primary health care needs. The medicinal plants play a major role in traditional system and constitute back bone of these systems [1]. Traditional medicine is the sum of the knowledge, skills, beliefs and experiences belonging to different cultures used in the maintenance of health as well as in the diagnosis, prevention, improvement or treatment of physical and mental illness. For the last decade, use of traditional medicine has expanded worldwide and has gained popularity [2]

Liver is the heaviest and largest gland of the body, weighing about 1.4 kg in an average adult. It is second largest organ of the body after the skin and is in the

Upper part of the abdominal cavity. Many drugs undergo various chemical changes in the liver before their excretion in bile or by other organs. They may damage the liver cells either in their original form or in various intermediate stages. Certain substances always cause liver damage.

Examples like chloroform, alcohol, ethanol, paracetamol, Diclofenac, etc.

### Diclofenac induced hepatotoxicity

Diclofenac is a proven, commonly prescribed nonsteroidal anti-inflammatory drug (NSAID) that has analgesic, anti-inflammatory and antipyretic properties, and has been shown to be effective in treating a variety of acute and chronic pain and inflammatory conditions. As with all NSAIDs, diclofenac exerts its action via inhibition of prostaglandin synthesis by inhibiting cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) with relative equipotency. However, extensive research shows the pharmacologic activity of diclofenac goes beyond COX inhibition, and includes multimodal and, in some instances, novel mechanisms of action (MOA).

MOAs of diclofenac; compares the drug's pharmacologic and pharmacodynamic properties with other NSAIDs to delineate its potentially unique qualities; hypothesizes why it has been chosen for further recent formulation enhancement; and evaluates the potential effect of its MOA characteristics on safety. Diclofenac can inhibit the thromboxane-prostanoid receptor, affect arachidonic acid release and uptake, inhibit lipoxygenase enzymes, and activate the nitric oxide-cGMP antinociceptive pathway. Other novel MOAs may include the inhibition of substrate P, inhibition of peroxisome proliferator activated receptor gamma (PPARgamma), blockage of acid-sensing ion channels, alteration of interleukin-6 production, and inhibition of N-methyl-D-aspartate (NMDA) receptor hyperalgesia.

Despite significant growth in hepatoprotective drugs, hunt for cost effective and less toxic agent in plants lasts to be a latent area of investigation. Evidence showed that many drug exhibiting hepatoprotective activity may produce its effect in different ways.

Nowadays use of plant preparations and extracts for hepatotoxicity has a long standing history among Indian physicians. Many indigenous plants having such activities are recorded in Ayurveda literature. Out of which, Turnera aphrodisiaca is one of the plant which has hepatoprotective activity. The present work deals with the evaluation of Turnerera aphrodisiaca leaf extract for hepatotoxicity activity. Turnera aphrodisiaca belongs to the family Turneceae commonly called as Damina or Old womens broom. It is mentioned in the literature that leaves are used in liver troubles, leaf boiled water as gargles, young leaves used as intermittent fever and for expelling worms. Though it is mentioned in the Ayurveda literature that it has hepatoprotective activity, no published scientific data is available. Hence an attempt has been made to evaluate the hepatoprotective activity of Turnera aphrodisiaca leaf extract in rats.

### Diclofenac induced hepatotoxicity [3-6]

Acute liver damage was induced in animals by the administration of Diclofenac (150 mg/kg. p.o) for 28 days. Treatment for 28 days.

Experimental design:

Animals were divided into 5 groups containing six animals each

I Group: Control group (2 % w/v acacia suspension).

II Group: Positive control group (Diclofenac, 150 mg/kg, p.o).

III Group: Standard group (100mg Silymarin, p.o) + Diclofenac (150 mg/kg, p.o).

IV Group: EETA (200 mg/kg, p.o) + Diclofenac, (150 mg/kg, p.o).

V Group: EETA (400 mg/kg, p.o) + Diclofenac, (150 mg/kg, p.o).

### Experimental Details

Group I was served as control, received acacia solution (2% w/v) in distilled water. Group II was maintained as positive control, received vehicle daily and diclofenac 150 mg/kg/b.w for 28 days. Group III was orally administered with standard silymarin once daily and diclofenac 150 mg/kg/b.w for 28 days. Group IV and V were orally administered with EETA at a dose of 200 & 400

mg/kg b.w respectively, once daily and diclofenac 150 mg/kg/b.w for 28 days.

On 29th day, 18 hours after the last dose of diclofenac, all the animals were anaesthetized and blood was collected from retro-orbital sinus in a heparinized capillary tube, and used for the estimation of SGPT (ALT), SGOT (AST), alkaline phosphatase (ALP), GGTP, total proteins, and total bilirubin.

On 29th day after collecting blood samples, animals were sacrificed by excess anesthetic agent. Liver were isolated and washed with ice cold saline solution and pressed between filter paper pads and weighed. A portion of each liver were used for estimation of LPO, SOD, CAT, GPX, GST, GSH and a portion of each liver were preserved in 10 % neutral formalin for histopathological studies.

## RESULTS AND DISCUSSION

### Diclofenac induced hepatotoxicity

Diclofenac treated group produced significant elevation of serum SGPT, SGOT, SALP, GGTP, total bilirubin and total proteins compared to the normal control group. However, EETA treated groups with 200 and 400 mg/kg, significantly decreased these serum biochemical indices as compared with diclofenac treated group. The liver weight of EETA treated group showed significantly decrease in both the doses when compare with positive control and where near to normal control.

The results of EETA treated groups on liver homogenate parameters such as LPO, SOD, CAT, GPX, GST and GSH showed significantly increase when compare with positive control.

Histopathological studies showed normal structure of hepatic lobules, while black spots are glycogen, white spots show the presence of vacuoles, a prominent blood vein with normal appearance in normal control group. Silymarin treated group showed normal structure same as normal control.Diclofenac treated group, showed marked damage of hepatic parenchyma, congestion and vesicular changes. Low dose of EETA treated group (200 mg/kg), showed recovery of hepatic parenchyma, mild congestion and microvesicular changes and high dose of EETA treated group (400 mg/kg), showed marked recovery in hepatic cells with nuclei, cytoplasm, central vein and portral traid when compare with positive control.

Diclofenac acts by inhibiting cellular cyclooxygenases (COX-1 and COX-2), which results in a decrease in production of pro-inflammatory prostaglandin, prostacyclin and thromboxane $A_2$ products, important mediators of inflammation and pain. Hepatotoxicity by diclofenac is typically associated with an acute hepatitis and necrosis that may be most prominent centrally and it usually produces focal necrosis and inflammation [7, 8].

Administration of diclofenac significantly ($P< 0.01$) elevated the SGPT, SGOT, SALP and GGTP activities compared to the normal animals. This marks the necrosis of hepatocytes resulting in the leakage of transaminases, GGTP and the elevation of serum markers. The significantly decreased serum markers and GGTP activities in the silymarin and EETA administered groups proved its hepatoprotective effect.

A large number of metabolites are produced by NSAIDs are found to generate superoxide anion and other free radicals in the biological systems. Treatment with EETA at both doses normalized the levels of enzymatic (SOD, CAT and GPx) and non-enzymatic (GSH and GST) antioxidants. Lipid peroxidation (LPO) increased after treatment with diclofenac reduced markedly after administration of silymarin and EETA at both doses and this further justified that the liver damage induced by diclofenac was restored. The liver damage induced by diclofenac was assessed by seeing the histopathology of liver sections. In extract treatment rats the level of histopathological damage was reduced and it was comparable to the effect of silymarin in bringing back normal hepatic architecture. Results are shown in tables

**Effect of ethanolic leaf extract Turnera aphrodisiaca on biochemical markers in diclofenac induced hepatotoxicity.**

| Group | Dose (mg/kg) | SGPT u/l | SGOT u/l | ALP u/l | Total Bilirubin (mg/dl) | GGTP u/l | TP mg/dl |
|---|---|---|---|---|---|---|---|
| Vehicle Control | 5 ml | 80±0.74 | 121±0.97 | 138±1.72 | 0.70±0.03 | 124±0.95 | 6.5±0.06 |
| Diclofenac | 150 | 372±0.58 | 356±2.95 | 410±0.08 | 2.42±0.17 | 254±2.65 | 7.4±0.08 |
| Silymarin + Diclofenac | 100 | 85±1.08 | 131±1.46 | 168±1.06 | 0.80±0.03 | 134±1.04 | 6.8±0.02 |
| EETA + Diclofenac | 200 | 88±1.75* | 132±1.39* | 184±1.30* | 0.80±0.02* | 130±1.05* | 6.9±0.04* |
| EETA + Diclofenac | 400 | 92±0.089** | 136±0.58** | 194±1.32** | 0.82±0.06** | 136±1.06** | 7.0±0.06** |

EETA- Ethanol extract of Turnera aphrodisiaca. All values are expressed as mean±SEM (n=6) one way ANOVA. *represents significant at p<0.05 and **represents highly significant at p<0.01 when compared with control.

**Effect of ethanolic extract Turnera aphrodisiaca leaf on liver weight in diclofenac induced hepatotoxicity.**

| **Group** | **Dose** (mg/kg) | **Liver weight (g) /100g of body weight** |
|---|---|---|
| Vehicle Control | 5 ml | 2.51 ± 0.42 |
| Diclofenac | 150 | 4.96 ± 0.73 |
| Silymarin + Diclofenac | 100 | 2.64 ± 0.18 |
| EETA + Diclofenac | 200 | 2.70 ±16* |
| EETA + Diclofenac | 400 | 3.2 ± 0.24** |

EETA- Ethanol extract of Turnera aphrodisiaca. All values are expressed as mean±SEM (n=6) one way ANOVA. *represents significant at p<0.05 and **represents highly significant at p<0.01 when compared with control.

**Effect of ethanolic extract of Turnera aphrodisiaca (EETA) leaf on liver homogenates parameters against Diclofenac induced injury in rats.**

| **Group** | **Dose (mg/kg)** | **LPO (mmol/mg)** | **SOD (U/mg)** | **CAT (U/mg)** | **GPX (U/mg)** | **GST (U/mg)** | **GSH (mmol/mg)** |
|---|---|---|---|---|---|---|---|
| Vehicle Control | 5 ml | 0.49±0.09 | 115.13±22.13 | 28.5±6.45 | 4.11±1.02 | 1.04±0.96 | 0.34±0.06 |
| Diclofenac | 150 | 4.14±1.04 | 39.25±10.12 | 10.34±5.14 | 1.98±0.95 | 0.55±0.09 | 0.01±0.03 |
| Silymarin + Diclofenac | 100 | 0.50±0.12 | 118.32±15.95 | 29.11±3.68 | 4.22±1.35 | 1.01±0.09 | 0.37±0.12 |
| EETA + Diclofenac | 200 | 0.66±0.14* | 120±12.87* | 28.2±3.15* | 4.12±1.09* | 1.26±0.59* | 0.38±0.03* |
| EETA + Diclofenac | 400 | 0.51±0.12** | 117.12±16.12** | 27.71±3.15** | 4.04±0.98** | 1.20±0.89** | 0.34±0.10** |

EETA- Ethanol extract of Turnera aphrodisiaca. All values are expressed as mean±SEM (n=6) one way ANOVA. *represents significant at p<0.05 and **represents highly significant at p<0.01 when compared with control.

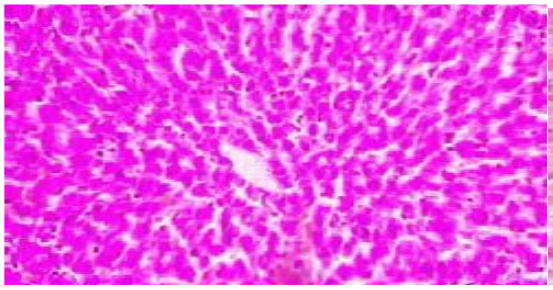

**Fig. a. Control**

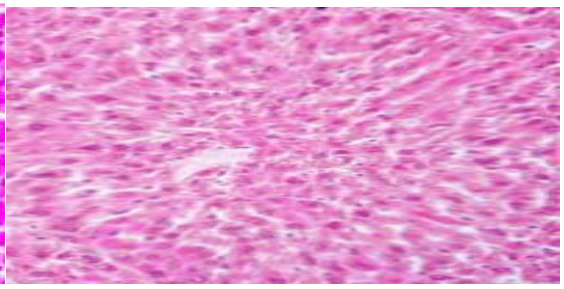

**Fig. b. Silymarin (100 mg/Kg)**

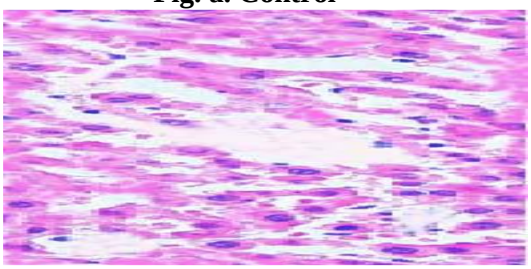

**Fig. c. Diclofenac (150 mg/kg)**

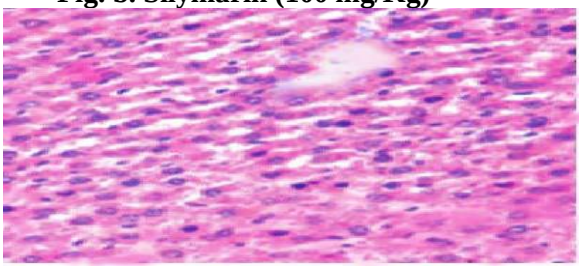

**Fig. d. EETA (200 mg/kg)**

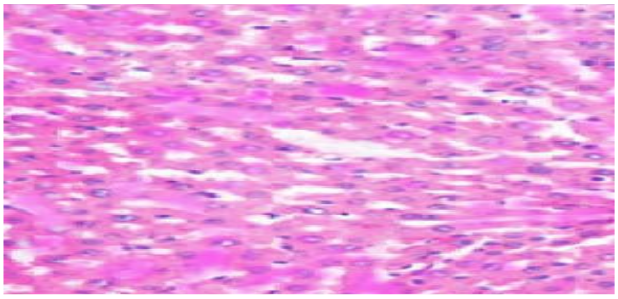

**Fig. e. EETA (400 mg/kg)**

**Histopathological studies of ethanolic extract of Turnera aphrodisiaca Leaf in diclofenac induced hepatotoxicity.**

**Fig.a.** Normal control group, showed normal structure of hepatic lobules, black spots are glycogen, white spots write down vacuoles, a prominent blood vein.

**Fig.b.** Silymarin treated group, showed normal structure of hepatic lobules, black spots are glycogen, white spots write down vacuoles, a prominent blood vein.

**Fig.c.** Diclofenac treated group, showed marked damage of hepatic parenchyma, congestion and vesicular changes.

**Fig.d.** EETA treated group (200 mg/kg), showed recovery of hepatic parenchyma, mild congestion and microvesicular changes.

**Fig.e.** EETA treated group (400 mg/kg), showed marked recovery in hepatic cells with nuclei, cytoplasm, central vein and portral traid.

## CONCLUSION

Diclofenac treated group produced significant elevation of serum SGPT, SGOT, SALP, GGTP, total bilirubin and total proteins compared to the normal control group. However, EETA treated groups with 200 and 400 mg/kg, significantly decreased these serum biochemical indices as compared with diclofenac treated group. The liver weight of EETA treated group showed significantly decrease in both the doses when compare with positive control and where near to normal control.

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