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Hepatoprotective effect of *Woodfordia fruticosa* Kurz flowers on diclofenac sodium induced liver toxicity in rats

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ABSTRACT

Objective: To evaluate the protective effect of *Woodfordia fruticosa* Kurz flowers against experimentally induced liver toxicity in rats. **Methods:** Two different doses of methanol extract of *Woodfordia fruticosa* (WFM) were evaluated for the hepatoprotective activity against diclofenac sodium induced hepatotoxicity in rats. Various biochemical parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), blood urea nitrogen (BUN) from serum; total protein (TP), glutathione (GSH) levels, catalase (CAT) and glutathione peroxidase (GPx) activities from liver were studied; histopathologic changes of liver were also evaluated. **Results:** WFM effectively reduced the elevated levels of serum ALT, AST, ALP and BUN, enhanced the reduced TP, ALB and hepatic GSH, CAT, GPx activity. The histopathological analysis suggested that WFM decreased the degree of liver fibrosis induced by diclofenac. **Conclusions:** This study demonstrates the hepatoprotective activity of WFM and thus scientifically support the use of this plant in traditional medicine for the treatment of liver disorders.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the centerpiece of pharmacotherapy for most rheumatological disorders, and are used in large numbers as analgesics and antipyretics, both as prescription drugs and over the counter purchases[1]. Diclofenac, a non-steroidal anti-inflammatory drug, was developed in the late 1970s and was approved for clinical use in treating several rheumatic diseases, and as an analgesic and anti-inflammatory agent. Like other non-steroidal anti-inflammatory drugs, long term use of diclofenac has been associated with a small but significant incidence of hepatotoxicity, ranging from mild, asymptomatic, reversible increase in liver function tests to jaundice and hepatitis, including several reports of fatal hepatitis[2,3].

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions[4]. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a major and important role in the management of various liver disorders[5].

Woodfordia fruticosa Kurz (syn. *Woodfordia floribunda* Salisb.) belongs to the family Lythraceae. Frequently used English names are Fire Flame Bush and Shiranjitea while in India (Gujarat) it is known as Dhavdi. All parts of this plant possess valuable medicinal properties *viz.* anti-inflammatory, anti-tumor, hepatoprotective and free radical scavenging activity[6,7], but flowers are in maximum demand[8]. In the present study, methanol extract of *Woodfordia fruticosa* Kurz. flowers was evaluated for its hepatoprotective property against diclofenac induced hepatotoxicity.

2. Materials and methods

2.1. Plant material

The fresh flowers of *Woodfordia fruticosa* were collected from Junagadh (Girnar region), Gujarat, India in the month of March 2009. The plant was compared with voucher specimen (voucher specimen No. PSN303) deposited by Dr. Nagar PS at Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India.

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2.2. Preparation of the extract

The flowers were washed under tap water, air dried, homogenized to fine powder and stored in airtight bottles. Ten grams of dried powder was first defatted with petroleum ether and then extracted with methanol by using Soxhlet apparatus. The solvent was evaporated to dryness and the dried crude extract was stored in air tight bottle at 4 ℃. The percentage yield of methanol extract was 36%. For testing, the *Woodfordia fruticosa* methanol (WFM) extract was dissolved in sterile distilled water and diluted to the desired concentrations.

2.3. Chemicals

All chemicals were of analytical-reagent grade and obtained from the following sources: Diclofenac sodium from Lekar Pharma Ltd. (Panoli, Bharuch, India); Silymarin from Micro Labs Ltd. (Solan, HP, India); Petroleum ether, methanol, acetic acid, hydrochloric acid from Merck (Darmstadt, Germany); Biochemical kits from Span Diagnostics Ltd (Sachin, Surat, India); Dipotassium hydrogen phosphate, potassiun dihydrogen orthophosphate, potassium dichromate, sodium citrate, hydrogen peroxide (H_2O_2), trichloroacetic acid (TCA), 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), sodium azide, glutathione reduced (GSH), tris free base were purchased from Himedia (Mumbai, India).

2.4. Animals

Wistar albino rats of either sex (180-220 g) were used for the study. The animals were obtained from Xcelris Labs Ltd., Ahmedabad. All the rats were kept in standard plastic rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were kept at the animal house of Department of Biosciences, Saurashtra University, Rajkot. The animals were facilitated with standard environmental condition of photoperiod (12:12 h dark: light cycle) and temperature [(25±2)℃] They were provided with commercial rat and mice feed (Pranav Agro Industries Ltd., Amruth Brand rat & mice pellet feed) and water given *ad libitum*. The use of these animals and the study protocols were approved by CPCSEA recognized local ethical committee.

2.5. Hepatoprotective study

The rats were divided into five groups with six rats in each. Group I (Control) served as normal and received the vehicle alone (Sterile distilled water 10 mL/kg body weight p.o.) for 5 days. Group II (Toxin control) animals received diclofenac (50 mg/kg i.p.) on the 3rd and 4th day. Group III (WFM-400) and IV (WFM-600) were treated with WFM at a dose level of 400 mg/kg and 600 mg/kg body weight p.o. per day, respectively for 5 days and on the 3rd and 4th day diclofenac (50 mg/kg i.p.) was given 1 h after the treatment of the extract. Group V (Standard) was treated with standard drug silymarin (100 mg/kg p.o.) for 5 days and on the 3rd and 4th day diclofenac (50 mg/kg i.p.) was given 1 h after the treatment of the drug. The animals were sacrificed 48 h after the last injection of diclofenac under mild ether anesthesia. The blood was collected and allowed to stand for 30 min at 37 ℃ and then centrifuged to separate the serum.

The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the liver was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged in a centrifuge at 5 000 rpm for 10 min, supernatant was collected and used for various antioxidant enzyme assays.

2.6. Estimation of biochemical parameters and antioxidant enzymes

The separated serum was estimated for various biochemical parameters like total protein, albumin, blood urea nitrogen, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels. Glutathione peroxidase and catalase activities and content of total protein and glutathione[9] was determined in the liver homogenate. The absorbance of all the parameters was measured in a UV-VIS Spectrophotometer (Shimadzu, Tokyo, Japan).

2.7. Histopathological study

A portion of the liver was cut into approximately 6 mm size and fixed in phosphate buffered 10% formaldehyde solution. After embedding in paraffin wax, thin sections of 5 μm thickness of liver tissue were cut and stained with hematoxylin-eosin. The thin sections of liver were made into permanent slides and examined under Zeiss microscope with photographic facility and photomicrographs were taken.

2.8. Statistical analysis

The data obtained from animal experiments are expressed as mean±SEM. Graphs were prepared using GraphPad Prism and data was subjected to analysis of variance (ANOVA) followed by Dunnett's *t*-test. Values are considered statistically significant at $P<0.05$.

3. Results

3.1. Serum biochemical analysis

The results of serum biochemical parameters in pre-treatment of WFM with respect to induction of hepatotoxicity using diclofenac sodium are shown in Figure 1. The level of total protein and albumin was depleted in the group treated with diclofenac sodium (toxin control) and they were significantly decreased ($P<0.001$) when compared with the normal control group. Serum ALT and AST levels were determined as an evaluation of hepatic function. The administration of diclofenac sodium markedly increased serum ALT and AST levels which were significant as

compared to normal control group (P<0.01, P<0.001, respectively). The ALP and blood urea nitrogen (BUN) levels also increased significantly (P< 0.001) in the group treated with diclofenac sodium.

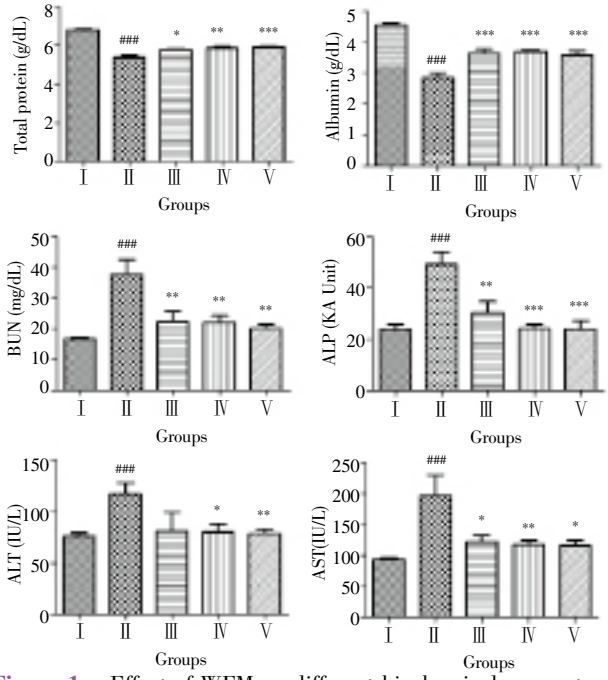

Figure 1. Effect of WFM on different biochemical parameters in diclofenac induced hepatic damage in rats.
Group I: Normal control, Group II: Toxin control diclofenac, Group III: WFM-400 mg/kg + diclofenac, Group IV: WFM-600 mg/kg + diclofenac, Group V: Silymarin-100 mg/kg + diclofenac. Results are expressed as mean ± SEM (n = 6). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ as compared with normal control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with toxin control group.

The groups that received the pre-treatment of WFM at dose levels of 400 and 600 mg/kg body weight significantly controlled the change in the biochemical parameters. The extract at dose levels of 400 and 600 mg/kg exhibited significant increase (P<0.05, P<0.01, respectively) in the serum total protein level as compared to toxin control group. The albumin level in lower as well as higher dose group increased significantly (P<0.001) as compared to toxin control group and the effect was comparable with the standard group (P<0.001) treated with silymarin. In WFM-600 group the level of ALT and AST significantly decreased (P<0.05), the result was comparable to the standard group. The ALP level significantly decreased in lower as well higher dose treated animals (P<0.001). The BUN level also decreased in both the dose groups significantly (P<0.01) as compared to toxin control group.

3.2. Liver antioxidants

The results of relative liver weight, liver total protein, GSH and antioxidant enzymes in diclofenac sodium induced hepatotoxicity are given in Figure 2. The relative liver weight in toxin control group increased significantly (P<0.001) as compared to normal control group. The total protein and GSH levels from the liver homogenate decreased significantly (P<0.001, P<0.05, respectively) in toxin control group. The catalase (CAT) and glutathione peroxidase (GPx) activity in the toxin control group was also found significantly (P<0.001, P<0.05, respectively) depleted as compared to the normal control group. The mean relative liver weight decreased significantly in WFM-400 and WFM-600 (P<0.001) treated group as compared to the toxin control group. The result of the higher dose group was comparable to the standard drug treated group (P<0.001). The total protein and GSH levels from liver homogenate in WFM treated groups elevated, but total protein level was not significant. However, pretreatment with WFM significantly recovered the diclofenac sodium induced GSH depletion in lower (P<0.01) and higher dose group. The catalase and GPx activity increased at both the dose levels. At higher dose WFM exhibited good activity (P<0.01, P<0.05, respectively). GPx activity of WFM-600 group was similar to that of standard drug treated group.

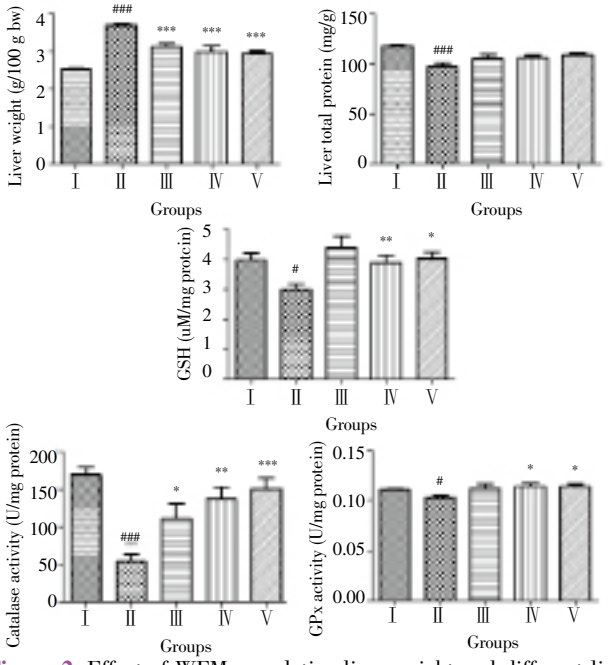

Figure 2. Effect of WFM on relative liver weight and different liver antioxidant enzymes in diclofenac induced hepatic damage in rats.
Group I: Normal control, Group II: Toxin control diclofenac, Group III: WFM-400 mg/kg + diclofenac, Group IV: WFM-600 mg/kg + diclofenac, Group V: Silymarin-100 mg/kg + diclofenac. Results are expressed as mean ± SEM (n = 6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ as compared with normal control group; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ as compared with toxin control group.

3.3. Histopathological changes

In normal control animal liver histological sections showed that hepatocytes were well-preserved, uniform cytoplasm and sinusoidal spaces (Figure 3A). Compared with the normal control group, liver tissue in the rats treated with diclofenac revealed extensive liver injuries, characterized by severe hepatocellular degeneration, necrosis, inflammatory cell infiltration, sinusoidal dilatation and cytoplasmic vacuolation (Figure 3B). However, the histopathological hepatic lesions induced by administration of diclofenac was remarkably improved by the treatment with WFM (Figure 3C & 3D), and showed marked protective effect by decreasing

hepatocellular degeneration and necrosis. The protective effect was also observed in silymarin treated animals (Figure 3E). This was in good agreement with the results of serum aminotransferase activity and hepatic oxidative stress level.

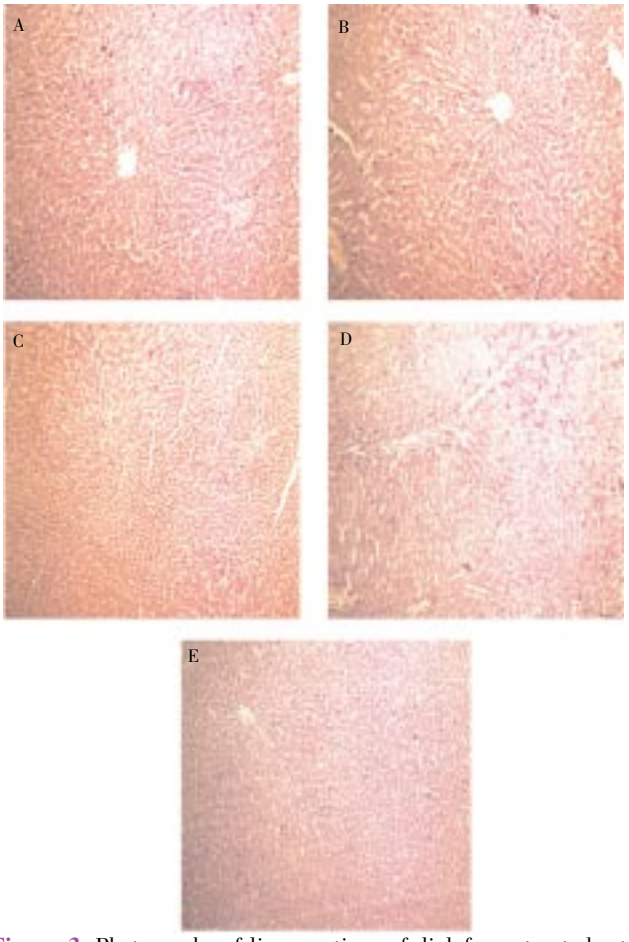

Figure 3. Photographs of liver sections of diclofenac treated rats (hematoxylin and eosin stained, 10×).
(A) Normal control group, (B) Toxin control (diclofenac) group, (C) WFM-400 mg/kg + diclofenac, (D) WFM-600 mg/kg + diclofenac, (E) Silymarin-100 mg/kg + diclofenac.

4. Discussion

Hepatic fibrosis is usually initiated by hepatocyte damage. Biologic factors such as hepatitis virus, bile duct obstruction, cholesterol overload, *etc*. or chemical factors such as CCl_4 administration, alcohol intake are known to contribute to liver fibrosis. The incidence of chronic fibrosis is high, but there have been no satisfactory agents with ascertained effectiveness and few side effects to liver. So, finding effective ways to inhibit liver fibrosis and prevent the development of cirrhosis are of great significance[10]. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms which have been disturbed by a hepatotoxic agent is the index of its protective effect[11].

Hepatotoxicity is currently a class warning for NSAIDs and infrequent hepatic injury has been observed for nearly all NSAIDs currently on the market, there are 3 drugs that have more commonly been associated with liver disease: diclofenac, sulindac, and aspirin[12]. Diclofenac sodium, undergoes similar hepatic metabolism both in rat and in humans. Major metabolic pathways are the hydroxylation in position 4 and 5 and to a much lesser extent the formation of 3'-hydroxy- (humans) and 4',5-dihydroxydiclofenac (rat and humans). Diclofenac and its metabolites undergo extensive conjugation with glucuronic acid and sulfate. The major constitutive P_{450} form involved in diclofenac hydroxylation in man is cytochrome P_{450}2C9, the human orthologous form of rat 2C11. Diclofenac forms selective protein adducts in livers of treated mice[13]. This is caused by a transacylation reaction of its glucuronide conjugate. This mechanism has been proposed to explain both the allergic and intrinsic hepatotoxicity of the drug.

Since unwanted side effects of diclofenac in man and other mammals were reported to occur particularly in the liver[14] it was thought of interest to evaluate WFM for their hepatoprotetive property in diclofenac induced hepatic damage in rats. In the present study, the administration of diclofenac to rats decreased the total protein and albumin level and increased the BUN level significantly. The pretreatment of WFM at two different dose levels restored the level of protein, albumin and BUN towards normalization. Hepatocellular injury from metabolic inhibition, oxygen radical toxicity, immunologically mediated damage, or some other mechanism results in predominant aminotransferase elevation, with elevations of alkaline phosphatase[15]. The ALT, AST and ALP levels significantly elevated when rats were administered with diclofenac indicating hepatocellular damage. The increased levels of these enzymes were significantly decreased by pretreatment with WFM in dose dependent manner. This is the indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by diclofenac sodium. The hepatoprotetive property of *Polyalthia longifolia* in diclofenac induced hepatic damage in rats was reported by Tanna *et al*[16].

The significant increased liver weight of diclofenac exposed animals seems to be due to toxic potential of diclofenac. The significant increase in weight of liver was, however, found to be associated with concomitant increase of serum AST and ALT enzyme levels. It is important to note that the elevated activity of serum AST and ALT recorded in this study may be due to loss of enzymes of liver tissue. Pretreatment of WFM decreased the liver weight significantly indicating recovery of liver tissue from damage. Significant decrease in total protein of the liver contents is a reflection of hepatic toxicity[17,18]. The significant reductions of protein in diclofenac intoxicated group indicate depletion in the protein reserve and thus suggest hepatic toxicity. WFM administration increased the total protein leading to normalization. GSH is an extremely efficient intracellular buffer for oxidative stress and GSH acts as a non-enzymatic antioxidant that reduces H_2O_2, hydroperoxides (ROOH) and xenobiotic toxicity[19-24]. The level of GSH depleted when animals were injected with diclofenac. The depleted level of GSH raised with the pretreatment of WFM. The catalase and GPx are enzymatic antioxidants widely distributed in all animal tissues that decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Therefore, the reduction in the activity of these two enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. In the present study, WFM significantly restored the hepatic catalase and GPx activity, which indicated that WFM could scavenge reactive free radicals that eventually lessen the oxidative damage to the tissues and subsequently

improved the activities of these antioxidant enzymes. The preventive effect of WFM was also confirmed by the results of histopathological examination, as evidenced by a dose related decrease in the incidence and severity of histopathological hepatic lesions.

WFM pretreatment prevented the reduction in the antioxidant enzyme activities and consequent oxidative damage to the liver. In fact, the multiple dose pretreatment of WFM alone significantly boosted the antioxidant enzyme activities. Similar results were also reported by Molina *et al*[25] and Scharf *et al*[26]. In conclusion, the results of this study demonstrate that the WFM was effective in prevention of diclofenac induced hepatic damage in rats. However, the hepatoprotective mechanisms of these medicinal herb constituents remain to be elucidated.

Conflict of interest statement

We declare that we have no conflict of interest.

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