



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(12)60310-4 ©2012 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Preventive and curative effect of *Woodfordia fruticosa* Kurz flowers on thioacetamide induced oxidative stress in rats

A Nitha, PN Ansil, SP Prabha, PJ Wills, MS Latha*

Biochemistry and Pharmacognosy Research Laboratory, School of Biosciences, Mahatma Gandhi University, P.D. Hills P.O, Kottayam, Kerala-686560, India

ARTICLE INFO

Article history:

Received 25 June 2012

Received in revised form 5 July 2012

Accepted 17 August 2012

Available online 28 August 2012

Keywords:

Woodfordia fruticosa

Thioacetamide

Oxidative stress

Hepatoprotection

Antioxidant

Silymarin

ABSTRACT

Objective: To evaluate the preventive and curative effect of *Woodfordia fruticosa* (*W. fruticosa*) Kurz flowers on thioacetamide induced oxidative stress in rats. **Methods:** Two different doses of methanolic extract of *W. fruticosa* (MEWF 100 mg/kg and 200 mg/kg) were used to study the antioxidant activity in experimental rats against thioacetamide (TAA) induced oxidative stress in preventive and curative models. Single dose of TAA (100 mg/kg; s.c.) was administered to the rats in all groups except the normal control. Various serum enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) were studied. The antioxidant status of liver and kidney were evaluated by the following parameters like catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), reduced glutathione (GSH) and malondialdehyde (MDA). Histopathological changes of liver tissue were also evaluated. **Results:** MEWF significantly ($P < 0.05$) prevented and reversed the elevation of serum AST, ALT, ALP, LDH, and tissue malondialdehyde levels in both the experimental models. Hepatic and renal GSH, GST, GR, GPx, and catalase levels were remarkably increased by the treatment with the extract in both the experimental models. In the case of MDA the hepatic and renal levels were decreased by the treatment with the extract. **Conclusion:** This study demonstrates the protective and curative effects of MEWF, and thus scientifically supports the use of this plant in traditional medicine for the treatment of liver disorders.

1. Introduction

Oxidative stress plays an important role in many diseases including those of the liver and it can be controlled by the antioxidant systems in living organisms[1]. Human systems possess enzymatic and non-enzymatic antioxidative mechanisms which minimizes the generation of reactive oxygen species[2]. When the generation of the active oxygen-free radical exceeds the scavenging ability many degenerative diseases such as brain dysfunction, cancer, heart diseases, age-related degenerative conditions, decline of the immune system, gastric ulcer and DNA damage will arise. Natural antioxidants present in fruits,

vegetables, cereals and medicinal plants act as effective free radical scavengers, by donating hydrogen to highly reactive radicals. Studies reveal that increased consumption of fruits rich in antioxidant polyphenols lower the risk of degenerative diseases[3]. Recently, interest in finding naturally occurring antioxidants has increased considerably to replace synthetic antioxidants such as butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) which are now used in drug composition. In food industry, the attention of manufactures has been shifted from synthetic to natural antioxidants due to their side effects.

Herbal drugs are playing an important role in health care programmes world wide, mainly due to the general belief that they are without any side effects besides being cheap and locally available. Lately there is resurgence of interest in herbal medicine for treatment of various ailments including liver disorders. In India, about 40 polyherbal commercial formulations with hepatoprotective action are being used. Hepatoprotective herbal drugs contain a variety

*Corresponding author: MS Latha, Biochemistry and Pharmacognosy Research Laboratory, School of Biosciences, Mahatma Gandhi University, P.D. Hills P.O, Kottayam, Kerala-686560, India.

Tel: +91 481 2731035; Fax: +91 481 2731009;

E-mail address: mslathasbs@yahoo.com

Grant number: KSCSTE Order No. (T) 298/SRS/2008/CSTE. Name of the supporting agency: Kerala State Council for Science Technology and Environment, Thiruvananthapuram, Kerala.

of chemical constituents like phenols, coumarins, lignins, essential oils, monoterpenes, carotenoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthenes[4]. *Woodfordia fruticosa* (*W. fruticosa*) kurz belongs to the family Lythraceae is a much branched beautiful shrub. All parts of the plant possess valuable medicinal properties viz anti-inflammatory, anti tumour, hepatoprotective and free radical scavenging activity[5,6] but flowers are in maximum demand. Dried flowers are used as tonic in disorders of mucous membrane, hemorrhoids and in derangement of liver. The phytochemical constituents isolated from dried flowers of *W. fruticosa* include octacosanol, β sitosterol, meso-inositol, ellagic acid, kaempferol etc[7]. In the present study the preventive and curative effect of MEWF was evaluated against TAA induced oxidative stress in rats.

2. Materials and methods

2.1. Chemicals

Thioacetamide (TAA) was purchased from Loba Cheme, Mumbai, India. Assay kits for serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and lactate dehydrogenase were purchased from Agappe Diagnostic, India. All other chemicals were of analytical grade.

2.2. Collection of plant material and preparation of plant extracts

W. fruticosa flowers were collected from natural habitat (Kollam, Kerala, India) and authenticated. A voucher specimen (SBSBRL.06) is maintained in the institute. Flowers were shade-dried and powdered. A 50 g of dried powder was soxhlet extracted with 400 mL of methanol for 48 h. The extracts were concentrated under reduced pressure using a rotary evaporator and were kept under refrigeration. The yield of methanolic extract was 12.5% (w/w). The concentrate was suspended in 5% Tween 80 for in vivo studies.

2.3. Animals and diets

Male Wistar rats weighing 150–160 g were used in this study. The animals were housed in polypropylene cages and given standard rat chow (Sai Feeds, Bangalore, India) and drinking water ad libitum. The animals were maintained at a controlled condition of temperature of 26–28 °C with a 12 h light: 12 h dark cycle. Animal studies were followed according to Institute Animal Ethics Committee regulations approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg. No. B 2442009/4) and conducted humanely.

2.4. Thioacetamide induced oxidative stress

Thioacetamide (TAA) suspended in normal saline was administered (100 mg/kg body weight) subcutaneously to induce the oxidative stress in rats[8]. Silymarin at an oral dose of 100 mg/kg body weight was used as standard control in the experiment[9]. Two different doses (100 and 200 mg/kg) of MEWF suspended in 5% Tween 80 were also prepared for oral administration to the animals. It is reported that the extracts of *W. fruticosa* flowers are safe with LD50 more than 2 000 mg/kg, p.o[5].

The animals were divided into pre-treatment and post-treatment groups. In each experiment five groups of animals were included. Group I was vehicle control, Group II was TAA control, Group III–V received silymarin at an oral dose of 100 mg/kg and MEWF at an oral dose of 100 and 200 mg/kg, b.w respectively. All the groups except group I received a single dose of TAA (100 mg/kg; s.c). Group I animals treated as vehicle control received 5% Tween 80 and normal saline instead of drug and TAA respectively.

2.4.1. Pre-treatment evaluation

In pre-treatment animals, all the groups except group I received a single dose of TAA (100 mg/kg; s.c) suspended in normal saline on 9th day of the experiment. Group III received silymarin and the groups IV and V received MEWF for 9 days before receiving TAA. Animals were sacrificed 24 h after TAA administration.

2.4.2. Post-treatment evaluation

In post-treatment animals, group II–V received TAA on the first day of the experiment. Groups III–V received silymarin and MEWF at 2, 24 and 48 h after TAA challenge. Animals were sacrificed 72 h after thioacetamide administration. Blood was collected from the neck blood vessels under mild ether anesthesia and kept for 30 min at 4°C. Serum was separated by centrifugation at 2 500 rpm at 4°C for 15 min. Dissected livers and kidneys were washed with normal saline and cut into separate portions for antioxidant estimation and for histopathological examination.

2.5. Serum enzyme analysis

Hepatotoxicity was assessed by quantifying the serum levels of AST (EC 2.6.1.1), ALT (EC 2.6.1.2), ALP (EC 3.1.3.1) and LDH (EC 1.1.1.27) by kinetic method using a standard diagnostic kit (Agappe Diagnostic Ltd., India). Activities of these serum enzymes were measured by using semi autoanalyzer (RMS, India).

2.6. Tissue analysis

Liver and kidney were excised, washed thoroughly in ice-cold saline to remove the blood. Ten percent of homogenate was prepared in 0.1 M Tris HCl buffer (pH 7.4). The homogenate was centrifuged at 3 000 rpm for 20 min at 4 °C and the supernatant was used for the estimation of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase

(GR), glutathione-S-transferase (GST), reduced glutathione (GSH), MDA (Thiobarbituric Acid Reactive Substances – TBARS) and total protein.

Tissue CAT (EC 1.11.1.6) activity was determined from the rate of decomposition of H_2O_2 [10]. GPx (EC 1.11.1.9) activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3 [11]. GR (EC 1.6.4.2) activity was assayed at 37 °C and 340 nm by following the oxidation of NADPH by GSSG[12]. GST (EC 2.5.1.18) activity was determined from the rate of increase in conjugate formation between reduced glutathione and CDNB[13]. GSH was determined based on the formation of a yellow colored complex with DTNB[14]. The level of lipid peroxidation was measured as malondialdehyde (MDA), a thiobarbituric acid reacting substance (TBARS), using 1'1'3'3' tetramethoxypropane as standard[15]. Protein content in the tissue was determined using bovine serum albumin (BSA) as the standard[16].

2.7. Histopathological studies

Small pieces of liver fixed in 10% buffered formalin were processed for embedding in paraffin. Sections of 5–6 μ m were cut and stained with hematoxylin and eosin and examined for histopathological changes under the microscope (Motic AE 21, Germany). The microphotographs were taken using Moticam 1000 camera at original magnification of 100 \times .

Liver sections were graded numerically to assess the degree of histological features. Acute hepatic injury is indicated by centrilobular necrosis – is the necrosis around the central vein characterized by prominent ballooning and swollen granular cytoplasm with fading nuclei. Bridging hepatic necrosis is a form of confluent necrosis of liver cells linking central veins to portal tracts or portal tracts to one another[17]. A combined score of centrilobular necrosis, bridging hepatic necrosis and lymphocyte infiltration was given a maximum value of 6 and descriptive modifiers such as mild, moderate, and severe was applied to activity and staging. The parameters were graded from score 0 to 6, with 0 indicating no abnormality, 1 to 2 indicating mild injury, 3 to 4 indicating moderate injury and 5 to 6 representing severe liver injury.

Table 1.

Antioxidant activity of WF methanolic extract on TAA induced biochemical changes in the liver of pre-treated animals ($n = 6$).

| Treatment groups | GSH (nmol/mg protein) | GST (μ mol CDNB–GSH conjugate formed/min/mg protein) | GR (nmol of GSSG utilized/min/mg protein) | GPx (nmol of GSH oxidized/min/mg protein) | CAT (U/mg protein) | MDA (nmol/g tissue) |
|--|-------------------------|---|---|---|-------------------------|-------------------------|
| Normal control | 23.5 \pm 0.6 | 68.5 \pm 0.7 | 17.3 \pm 0.5 | 291.4 \pm 6.2 | 49.8 \pm 2.3 | 46.2 \pm 0.7 |
| Thioacetamide (100 mg/kg ;S.C) | 13.9 \pm 0.5 Δ | 32.4 \pm 0.5 Δ | 6.2 \pm 0.6 Δ | 167.2 \pm 5.9 Δ | 31.9 \pm 1.4 Δ | 78.7 \pm 1.1 Δ |
| Silymarin (100 mg/kg)+Thioacetamide | 20.2 \pm 0.4* | 57.5 \pm 0.8* | 13.6 \pm 0.3* | 262.6 \pm 7.4* | 44.5 \pm 1.9* | 55.2 \pm 0.8* |
| <i>W. fruticosa</i> (100 mg/kg) +Thioacetamide | 17.2 \pm 0.6* | 43.5 \pm 0.6* | 11.6 \pm 0.4* | 210.5 \pm 6.0* | 38.7 \pm 1.0* | 64.8 \pm 1.0* |
| <i>W. fruticosa</i> (200 mg/kg) +Thioacetamide | 22.3 \pm 0.4* | 65.9 \pm 0.4* | 16.2 \pm 0.3* | 278.5 \pm 5.3* | 48.1 \pm 1.5* | 48.7 \pm 0.6* |

$\Delta P < 0.05$ versus normal control. * $P < 0.05$ versus thioacetamide control. Values are mean \pm S.D.

2.8. Statistical analysis

Results were expressed as mean \pm SD and all statistical comparisons were made by means of one-way ANOVA test followed by Tukey's post hoc analysis and P -values less than or equal to 0.05 were considered significant.

3. Results

3.1. Serum analysis

3.1.1. Pre-treatment evaluation

The serum levels of AST, ALT, ALP and LDH in group II were significantly ($P < 0.05$) elevated by the administration of a single dose of TAA, when compared to normal control. The treatment of methanolic extract of WF at a dose of 100 and 200 mg/kg showed a significant decrease ($P < 0.05$) of AST, ALT, ALP and LDH. Standard control drug, silymarin at a dose of 100 mg/kg also prevented the elevation of serum enzymes (Figure 1). Treatment with 200 mg/kg methanolic extract and silymarin exhibited a protection of 96.5% and 72.3% in AST levels, 97.5% and 67.8% in ALT levels, 98.2% and 62.2% in ALP levels and 90.5% and 68.4% in LDH levels, respectively. The preventive effect of the extract in decreasing the elevated levels of serum enzymes was in a dose dependent manner.

3.1.2. Post-treatment evaluation

There was a significant ($P < 0.05$) rise in the serum levels of AST, ALT, ALP and LDH after administration of TAA in post-treated animals. In contrast, treatment with methanolic extract of (100 and 200 mg/kg) WF exhibited an ability to counteract the TAA induced hepatotoxicity by decreasing the serum enzymes levels ($P < 0.05$) compared to TAA control. 200 mg/kg of methanolic extract showed a protection of 96.8%, 98.0%, 98.6% and 91.5% for AST, ALT, ALP and LDH respectively. Silymarin also showed a remarkable protection of 81.9%, 80.4%, 70.2% and 79.4% for AST, ALT, ALP and LDH respectively towards TAA intoxication ($P < 0.05$) (Figure 1).

3.2. Antioxidant activity of WF on TAA induced biochemical changes in the liver and kidney of pre and post treated animals

Table 2.Antioxidant activity of WF methanolic extract on TAA induced biochemical changes in the kidney of pre-treated animals ($n = 6$).

| Treatment groups | GSH (nmol/mg protein) | GST (μ mol CDNB–GSH conjugate formed/min/mg protein) | GR (nmol of GSSG utilized/min/mg protein) | GPx (nmol of GSH oxidized/min/mg protein) | CAT (U/mg protein) | MDA (nmol/g tissue) |
|--|------------------------|---|---|---|-------------------------|-------------------------|
| Normal control | 17.5 \pm 0.6 | 48.6 \pm 0.7 | 15.4 \pm 0.3 | 278.4 \pm 7.1 | 55.7 \pm 0.9 | 41.8 \pm 0.3 |
| Thioacetamide (100 mg/kg; S.C) | 8.4 \pm 0.3 Δ | 25.2 \pm 0.5 Δ | 5.6 \pm 0.5 Δ | 152.3 \pm 6.3 Δ | 41.5 \pm 1.2 Δ | 74.6 \pm 0.8 Δ |
| Silymarin (100 mg/kg)+Thioacetamide | 15.2 \pm 0.4* | 43.1 \pm 0.3* | 12.4 \pm 0.6* | 243.5 \pm 4.6* | 51.8 \pm 1.9* | 52.4 \pm 0.5* |
| <i>W. fruticosa</i> (100 mg/kg) +Thioacetamide | 12.7 \pm 0.4* | 38.6 \pm 0.6* | 10.2 \pm 0.4* | 189.5 \pm 6.9* | 47.2 \pm 1.5* | 61.1 \pm 0.7* |
| <i>W. fruticosa</i> (200 mg/kg) +Thioacetamide | 16.8 \pm 0.5* | 47.9 \pm 0.4* | 14.9 \pm 0.3* | 267.3 \pm 4.8* | 53.4 \pm 0.7* | 45.6 \pm 0.5* |

 $\Delta P < 0.05$ versus normal control. * $P < 0.05$ versus thioacetamide control. Values are mean \pm S.D.**Table 3.**Antioxidant activity of WF methanolic extract on TAA induced biochemical changes in the liver of post – treated animals ($n = 6$).

| Treatment groups | GSH (nmol/mg protein) | GST (μ mol CDNB–GSH conjugate formed/min/mg protein) | GR (nmol of GSSG utilized/min/mg protein) | GPx (nmol of GSH oxidized/min/mg protein) | CAT (U/mg protein) | MDA (nmol/g tissue) |
|--|-------------------------|---|---|---|-------------------------|-------------------------|
| Normal control | 24.7 \pm 0.6 | 73.7 \pm 0.3 | 20.6 \pm 0.5 | 284.7 \pm 6.2 | 48.9 \pm 1.8 | 47.5 \pm 1.2 |
| Thioacetamide (100 mg/kg; S.C) | 14.5 \pm 0.4 Δ | 36.8 \pm 0.6 Δ | 8.3 \pm 0.4 Δ | 169.6 \pm 7.5 Δ | 35.8 \pm 1.2 Δ | 72.6 \pm 0.8 Δ |
| Silymarin (100 mg/kg)+Thioacetamide | 19.2 \pm 0.7* | 60.5 \pm 0.5* | 15.8 \pm 0.8* | 253.8 \pm 5.7* | 42.9 \pm 1.5* | 55.6 \pm 0.8* |
| <i>W. fruticosa</i> (100 mg/kg) +Thioacetamide | 17.3 \pm 0.5* | 57.6 \pm 0.8* | 12.9 \pm 0.6* | 214.1 \pm 5.9* | 38.6 \pm 1.6* | 61.3 \pm 1.4* |
| <i>W. fruticosa</i> (200 mg/kg) +Thioacetamide | 22.9 \pm 0.4* | 69.2 \pm 0.4* | 19.6 \pm 0.5* | 278.6 \pm 4.8* | 47.4 \pm 1.3* | 49.8 \pm 0.6* |

 $\Delta P < 0.05$ versus normal control. * $P < 0.05$ versus thioacetamide control. Values are mean \pm S.D.**Table 4.**Antioxidant activity of WF methanolic extract on TAA induced biochemical changes in the kidney of post-treated animals ($n = 6$).

| Treatment Groups | GSH (nmol/mg protein) | GST (μ mol CDNB–GSH conjugate formed/min/mg protein) | GR (nmol of GSSG utilized/min/mg protein) | GPx (nmol of GSH oxidized/min/mg protein) | CAT (U/mg protein) | MDA (nmol/g tissue) |
|--|------------------------|---|---|---|-------------------------|-------------------------|
| Normal control | 18.6 \pm 0.5 | 47.8 \pm 0.8 | 17.1 \pm 0.4 | 274.7 \pm 6.0 | 56.4 \pm 1.3 | 42.3 \pm 0.7 |
| Thioacetamide (100 mg/kg; S.C) | 9.1 \pm 0.4 Δ | 28.5 \pm 0.6 Δ | 7.3 \pm 0.6 Δ | 169.6 \pm 4.8 Δ | 44.8 \pm 1.5 Δ | 70.8 \pm 0.8 Δ |
| Silymarin (100 mg/kg)+Thioacetamide | 14.6 \pm 0.7* | 40.6 \pm 1.1* | 14.1 \pm 0.5* | 248.1 \pm 5.4* | 51.4 \pm 0.9* | 52.5 \pm 0.5* |
| <i>W. fruticosa</i> (100 mg/kg) +Thioacetamide | 12.8 \pm 0.8* | 36.4 \pm 0.9* | 11.6 \pm 0.4* | 211.4 \pm 6.2* | 48.2 \pm 1.4* | 59.7 \pm 0.8* |
| <i>W. fruticosa</i> (200 mg/kg) +Thioacetamide | 17.2 \pm 0.6* | 45.8 \pm 0.5* | 16.5 \pm 0.3* | 267.5 \pm 4.5* | 55.1 \pm 1.1* | 45.4 \pm 0.6* |

 $\Delta P < 0.05$ versus normal control. * $P < 0.05$ versus thioacetamide control. Values are mean \pm S.D.

3.2.1. Estimation of reduced glutathione (GSH)

In the pre-treatment groups, rats administered with TAA alone were found significantly ($P < 0.05$) lowered level of reduced glutathione (GSH). Treatment with methanolic extract exhibited significant increase ($P < 0.05$) in both hepatic (Table 1) and renal (Table 2) glutathione levels. In liver and kidney, 200 mg/kg of methanolic extract showed a protection of 87.5% and 92.3% respectively. Silymarin-treated rats also showed considerable prevention of GSH and the percentage of protection was 65.6% and 74.7% respectively in liver and kidney.

In the post-treatment groups, rats treated with 100 and 200 mg/kg methanolic extract significantly ($P < 0.05$) restored the decreased glutathione levels in liver (Table 3) and kidney (Table 4). The results were comparable with silymarin. In hepatic tissue, 82.3% reversal in GSH level shown by 200 mg/kg of methanolic extract was comparable with 46% exhibited by 100 mg/kg of silymarin. In renal tissue, 200 mg/kg of methanolic extract and 100 mg/kg of silymarin restored the GSH level by 85.2% and 57.8% respectively.

3.2.2. Estimation of glutathione – S – transferase (GST)

The GST activity of liver and kidney tissues were significantly ($P < 0.05$) reduced in TAA intoxicated rats of pre-treatment groups compared to normal control. The WF methanolic extract dose dependently increased ($P < 0.05$) the activity of GST in both the hepatic and renal tissues (Table 1 & 2). Treatment with 200 mg/kg methanolic extract exhibited prominently increased *i.e.*, 92.7% and 97%, respectively in hepatic and renal GST levels. In addition, Silymarin treated rats also prevented the TAA induced decrease in GST activity by 69.5% and 76.4% in hepatic and renal tissues respectively.

Rats administered with TAA alone showed significant ($P < 0.05$) reduction in hepatic and renal GST level in post-treatment groups. Treatment with methanolic extract of WF at a dose of 100 mg/kg and 200 mg/kg showed significant reversal ($P < 0.05$) of TAA induced toxicity. Silymarin (100 mg/kg) also markedly ($P < 0.05$) inhibited the TAA induced decrease in GST activity. Rats treated with 200 mg/kg methanolic extract and 100 mg/kg silymarin restored the decrease of GST levels by 87.8% and 63.4% in the liver and 89.6% and 62.6% in the kidney respectively (Table 3 & 4).

3.2.3. Estimation of glutathione reductase (GR)

GR activity was significantly decreased ($P<0.05$) in TAA treated animals when compared to control in the pre-treatment groups. A significant increase ($P<0.05$) in the level of GR was observed in WF methanolic extract (100 and 200 mg/kg) and silymarin (100 mg/kg) treated rats intoxicated with TAA. Both hepatic and renal tissues showed the same pattern of GR activity in all groups treated with WF methanolic extract and silymarin (Table 1 & 2). The percentage of protection in liver and kidney were 86.9% and 94.8% respectively for 200 mg/kg of methanolic extract. Silymarin restored the GR activity upto 66.6% in liver and 69.3% in kidney.

In the post-treatment groups, rats administered with TAA alone significantly ($P<0.05$) reduced the activity of glutathione reductase. Treatment with methanolic extract

exhibited significant increase ($P<0.05$) in both hepatic (Table 3) and renal (Table 4) GR activity. In liver and kidney, 200 mg/kg of methanolic extract restored the activity of GR by 91.8% and 93.8% respectively. Silymarin-treated rats also restored GR activity upto 60.9% and 69.3% in liver and kidney.

3.2.4. Estimation of glutathione peroxidase (GPx)

Activities of hepatic and renal GPx in pre-treatment groups were significantly ($P<0.05$) lowered in TAA treated rats (Table 1 & 2). WF methanolic extract dose dependently prevented the lowering of GPx in both the organs compared to TAA alone treated groups. In liver and kidney, 200 mg/kg of methanolic extract showed a protection of 89.6% and 91.1% respectively. Silymarin-treated rats also prevented the lowering of GPx by 76.8% in hepatic and 87.4% in renal tissues.

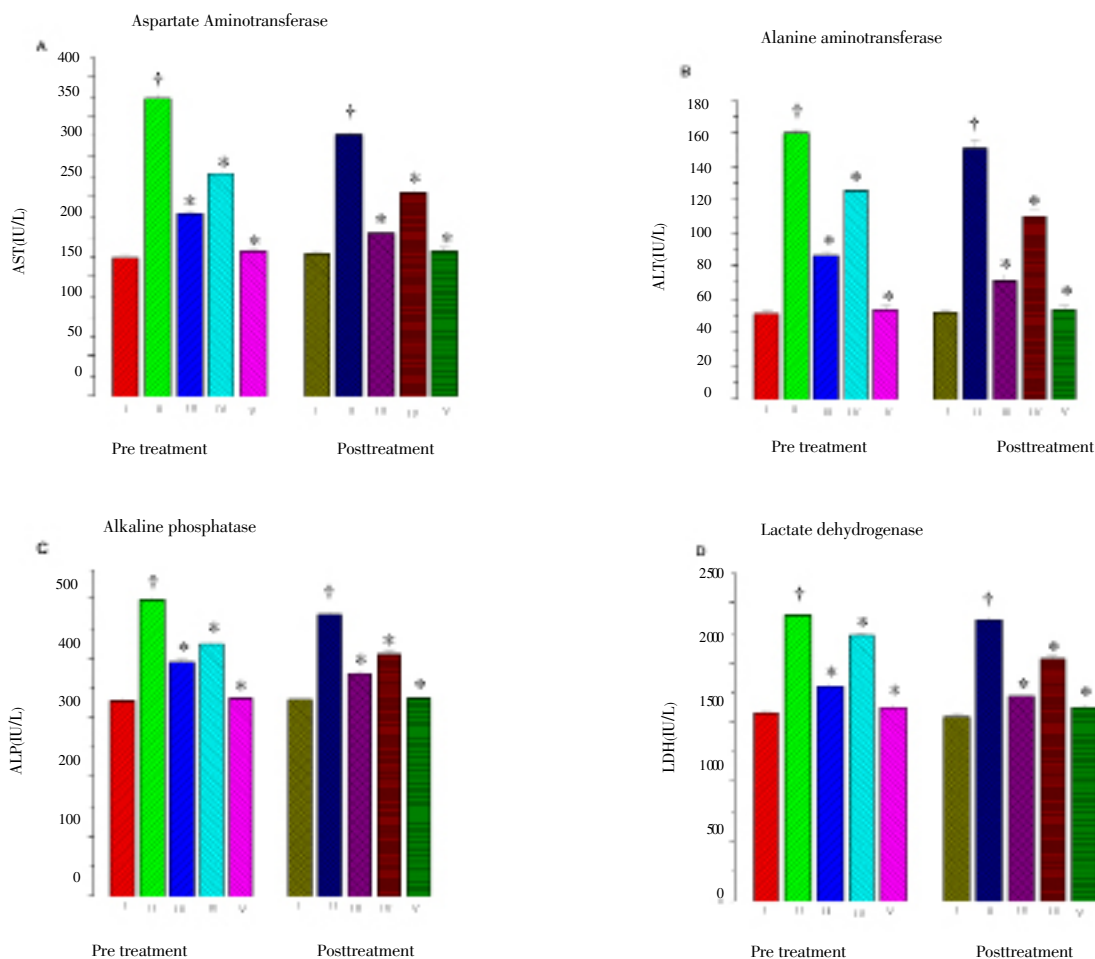


Figure 1. Effects of MEWF on changes in serum enzyme levels of rats treated with TAA. (A) Aspartate aminotransferase, (B) Alanine aminotransferase, (C) Alkaline phosphatase and (D) Lactate dehydrogenase. (I) Normal control, (II) Thioacetamide control, (III) Silymarin, (IV) MEWF – 100 mg/kg, (V) MEWF – 200 mg/kg. Values are mean \pm S.D, error bar indicating the standard deviation, $n = 6$. † $P<0.05$ vs. normal control. * $P<0.05$ vs. Thioacetamide control.

In the post-treatment groups, rats treated with 100 and 200 mg/kg methanolic extract significantly ($P < 0.05$) restored the decreased GPx activity in liver (Table 3) and kidney (Table 4). In hepatic tissue, 94.7% reversal in GPx activity shown by 200 mg/kg of methanolic extract was comparable with 73.1% exhibited by 100 mg/kg of Silymarin. In renal tissue, 200 mg/kg of methanolic extract and 100 mg/kg of Silymarin reinstated the GPx activity by 92.7% and 74.6% respectively.

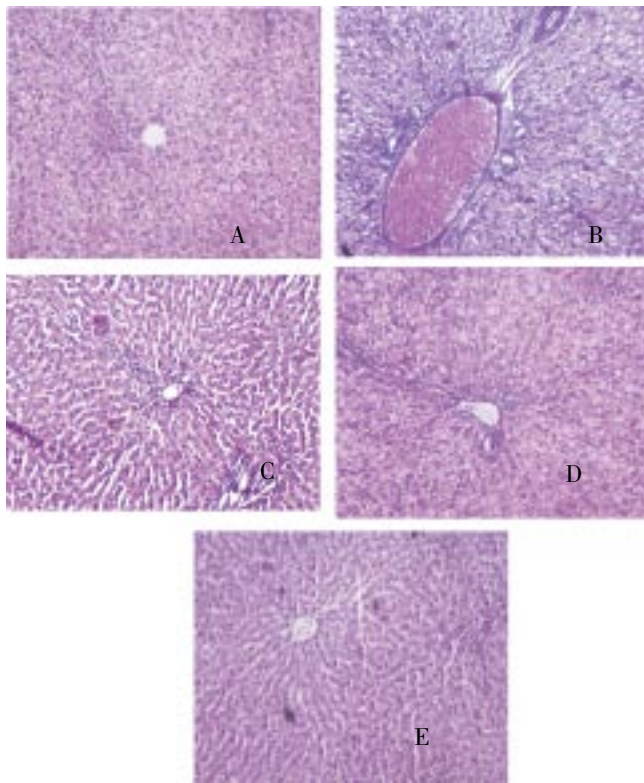


Figure 2. Histopathological changes occurred in rat liver due to pre-treatment with MEWF (hematoxylin and eosin, 100 \times). (A) Normal control; (B) TAA control (100 mg/kg s.c.); (C) Silymarin (100 mg/kg) + TAA; (D) MEWF (100 mg/kg) + TAA; (E) MEWF (200 mg/kg) + TAA.

3.2.5. Estimation of catalase (CAT)

The CAT activity in liver and kidney showed a significant ($P < 0.05$) reduction in TAA intoxicated rats of pre-treatment groups compared to normal control. The WF methanolic extract dose dependently increased the activity of CAT in both hepatic and renal tissues (Table 1 & 2). Treatment with 200 mg/kg methanolic extract exhibited significant increase *i.e.*, 90.5% and 83.8%, respectively in liver and kidney. In addition, silymarin treated rats also prevented ($P < 0.05$) the TAA induced decrease in CAT activity by 70.3% and 72.5% in hepatic and renal tissues respectively.

Animals injected with TAA alone showed significant ($P < 0.05$) reduction in hepatic and renal CAT activity in post-treatment groups. Treatment with methanolic extract of WF at a dose of 100 mg/kg and 200 mg/kg showed significant reversal ($P < 0.05$) of TAA induced hepatotoxicity. Silymarin (100 mg/kg) also markedly ($P < 0.05$) restored the TAA induced decrease in CAT activity. Rats treated with 200 mg/kg methanolic extract and 100 mg/kg silymarin restored the decrease of CAT levels by 88.5% and 54.1% in the liver and

88.7% and 56.8% in the kidney respectively (Table 3 & 4).

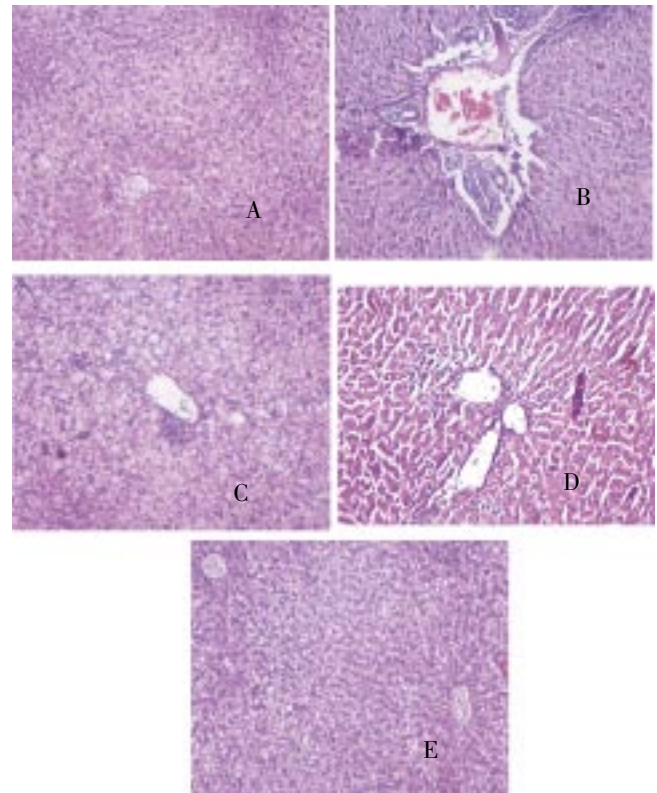


Figure 3. Histopathological changes occurred in rat liver due to post-treatment with MEWF (hematoxylin and eosin, 100 \times). (A) Normal control; (B) TAA control, (100 mg/kg s.c.); (C) TAA + Silymarin (100 mg/kg); (D) TAA + MEWF (100 mg/kg); (E) TAA + MEWF (200 mg/kg).

3.2.6. Estimation of lipid peroxidation (MDA)

In pre-treatment animals, a significant increase ($P < 0.05$) in tissue MDA level was observed in TAA alone treated rats. However, TAA induced elevation of MDA concentration were lowered ($P < 0.05$) by 92.3% in hepatic and 88.4% in renal tissues of rats treated with WF methanolic extract at a dose of 200 mg/kg. Silymarin also showed a protection ($P < 0.05$) of 72.3% in liver and 67.6% in kidney (Table 1 & 2).

In post-treatment animals (Table 3 & 4), a significant increase ($P < 0.05$) in tissue MDA level was shown in TAA alone treated animals when compared to normal control. WF methanolic extract and silymarin significantly ($P < 0.05$) reversed the elevation of hepatic and renal MDA formation. 200 mg/kg of WF methanolic extract reinstated the MDA formation by 90.8% in hepatic tissue and 89.1% in renal tissue. Silymarin exhibited 67.7% and 71.7% inhibition in MDA formation in liver and kidney respectively.

3.3. Histopathological analysis

In pre-treatment groups, rats treated with TAA, the normal architecture of liver (Figure 2B) was completely lost with the appearance of centrilobular necrosis, bridging hepatic necrosis and lymphocyte infiltration with a score of 5.4 ± 0.4 (mean \pm S.D.; $n=3$). The animals administered with silymarin and methanolic extract at 100 and 200 mg/kg showed a significant ($P < 0.05$) protection from TAA induced liver damage as evident from hepatic architectural pattern

with mild to moderate hepatitis with scores 2.4 ± 0.6 ; 2.8 ± 1.0 ; and 2.0 ± 0.5 (mean \pm S.D.; $n=3$; $P<0.05$), respectively (Figure 2C–E).

In the post-treatment group, TAA intoxicated rats showed a maximum score of 5.2 ± 0.8 (mean \pm S.D.; $n=3$) (Figure 3B). Rats treated with silymarin and WF methanolic extract (100 and 200 mg/kg) after the establishment of toxic injury showed recovery from centrilobular necrosis, bridging necrosis and lymphocyte infiltration with scores 1.8 ± 0.6 ; 2.2 ± 0.4 and 1.1 ± 0.5 (Mean \pm S.D.; $n=3$; $P<0.05$), respectively (Figure 3C–E).

4. Discussion

Thioacetamide (TAA) is a compound endowed with liver damaging and carcinogenic activity. It has been used to induce a model of acute liver injury in rats^[18]. Shortly after its administration, thioacetamide is metabolized to acetamide and thioacetamide-5-oxide. The latter binds to tissue macromolecules and is responsible for the change in cell permeability, increased intracellular concentration of Ca^{2+} , increase in nuclear volume and enlargement of nucleoli and inhibits mitochondrial activity eventually leading to hepatic necrosis^[19].

The increase in the activities of AST, ALT, ALP and LDH in serum of rats treated with TAA might be due to the increased permeability of plasma membrane or cellular necrosis leading to leakage of the enzymes to the blood stream^[20] and this showed the stress condition of the TAA treated animals. In the present study, administration of a single dose of TAA elevated the levels of serum AST, ALT, ALP and LDH in the untreated toxic control. In preventive and curative models a marked reduction of serum AST, ALT, ALP and LDH levels was observed in rats treated with MEWF. The extract at 200 mg/kg produced better results than 100 mg/kg, shows the dose response action of the extract. These results demonstrate the preventive and curative effect of MEWF against TAA intoxication.

Generation of a large amount of ROS due to TAA can overwhelm the antioxidant defense mechanism and damage cellular ingredients such as lipids, proteins and DNA; this in turn can impair cellular structure and function. The intra cellular antioxidant system comprises of different free radical scavenging antioxidant enzymes along with some non-enzyme antioxidants like GSH, CAT, GST, GPx, and GR constitute the first line of cellular antioxidant defense enzymes. When excess free radicals are produced, the equilibrium is lost and consequently oxidative insult is established^[21]. Glutathione detoxifies toxic metabolites of drugs, regulates gene expression, apoptosis and transmembrane transport of organic solutes and it is essential to maintain the reduced status of the cell/tissue. Hepatic and renal damage induced by TAA administration is associated with cellular necrosis, increase in tissue lipid

peroxidation and depletion in the tissue GSH, GST, GR, GPx and CAT activities.

In the present study, treatment with MEWF and silymarin significantly ($P<0.05$) enhanced the hepatic and renal GSH, GST, GR, GPx and CAT level compared to the TAA alone treated animals. This could explain the dose dependent (200 and 100 mg/kg) preventive and curative action of the extract. Pre and post-treatment with MEWF significantly ($P<0.05$) enhanced the GST activity, may be due to the decreased bioactivation of TAA. GST offers protection against lipid peroxidation by promoting the conjugation of toxic electrophiles with GSH^[22]. GR is also essential for the maintenance of GSH levels *in vivo*. The significant ($P<0.05$) restoration of GPx activity in MEWF and silymarin in pre and post-treated rats might be due to the antioxidant activity by detoxifying the endogenous metabolic peroxides generated after TAA injury in hepatic and renal tissues. Catalase is responsible for the breakdown of H_2O_2 , an important ROS, formed during the reaction catalyzed by SOD^[23]. Reduced activity of CAT after exposure to TAA in the present finding could be correlated to increased generation of H_2O_2 . The pre and post-treatment of MEWF significantly ($P<0.05$) aided to maintain the CAT activity near to normal level in both hepatic and renal tissues. This evidently shows the antioxidant property of the extract against oxygen free radicals.

MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation^[24]. The concentration of MDA in tissues of TAA alone exposed group was significantly ($P<0.05$) differed from that of normal control. Pre and post-treatment of rats with MEWF protected the liver and kidney from increased MDA formation. This demonstrates the antilipid peroxidative effect of the extract. Histopathological evaluation showed negligible damage to a few hepatocytes present in the close vicinity of central vein in MEWF treated rats and the improvement of histological scores proved the efficacy of the extract as an antihepatotoxic agent.

MEWF contains β -sitosterol, a component reported as a hepatoprotective agent^[25] and ellagic acid, a strong antioxidant and chemoprotective agent^[7]. The identified class of components in single or in combination with other components present in the extract might be responsible for the antihepatotoxic activity in both the treatment groups. In conclusion, the result of serum biochemical parameters, level of hepatic and renal lipid peroxides, glutathione antioxidant systems, CAT and histopathological studies support the dose dependent hepatoprotective and antioxidant activity of MEWF. The present study also supports the traditional use of WF flowers in derangement of liver. So this can be employed as main ingredient in medicine for disorders due to oxidative stress. However, further pharmacological evidences at molecular level are required to establish the mechanism of action of the drug

which is underway.

Conflict of interest statement

The authors declare that are no conflicts of interest.

Acknowledgments

Authors are thankful to Mr. Jayesh Kuriakose, Research scholar, SBS, M. G. University, Kottayam for his help in this work.

References

- [1] Ravikumar S, Gnanadesigan M. Hepatoprotective and antioxidant activity of a mangrove plant *Lumnitzera racemosa*. *Asian Pac J Trop Biomed* 2011; **1**(5): 348–352.
- [2] Sinha D, Roy S, Roy M. Antioxidant potential of tea reduces arsenite induced oxidative stress in Swiss albino mice. *Food Chem Toxicol* 2010; **48**: 1032–1039.
- [3] Patel DK, Kumar R, Prasad SK, Hemalatha S. *Pedaliium murex* Linn (Pedaliaceae) fruits: a comparative antioxidant activity of its different fractions. *Asian Pac J Trop Biomed* 2011; **1**(5): 395–400.
- [4] Eesha BR, Mohanbabu Amberkar V, Meena Kumari K, Sarath B, Vijay M, Lalit M, et al. Hepatoprotective activity of *Terminalia paniculata* against paracetamol induced hepatocellular damage in Wistar albino rats. *Asian Pac J Trop Med* 2011; **4**(6):466–469
- [5] Chandan BK, Saxena AK, Shukla S, Sharma N, Gupta DK, Singh K, et al. Hepatoprotective activity of *Woodfordia fruticosa* Kurz flowers against carbon tetrachloride induced hepatotoxicity. *J Ethnopharmacol* 2008; **119**(2): 218–224.
- [6] Yogesh Baravalia, Yogeshkumar Vaghasiya, Sumitra Chanda. Hepatoprotective effect of *Woodfordia fruticosa* Kurz flowers on diclofenac sodium induced liver toxicity in rats. *Asian Pac J Trop Med* 2011; **4**(5): 342–346.
- [7] Das PK, Goswami S, Chinniah A, Panda N, Banerjee S, Sahu NP, et al. *Woodfordia fruticosa*: Traditional uses and recent findings. *J Ethnopharmacol* 2007; **110**(2): 189–199.
- [8] Devaraj VC, Krishna BG, Viswanatha GL, Kamath JV, Kumar S. Hepatoprotective activity of Hepax–A polyherbal formulation. *Asian Pac J Trop Biomed* 2011; **1**(2): 142–146.
- [9] Shyamal S, Latha PG, Suja SR, Shine VJ, Anuja GI, Sini S, et al. Hepatoprotective effect of three herbal extracts on aflatoxin B1–intoxicated rat liver. *Singapore Med J* 2010; **51**(4): 326–331.
- [10] El–Demerdash FM, Yousef MI, Radwan FM. Ameliorating effect of curcumin on sodium arsenite–induced oxidative damage and lipid peroxidation in different rat organs. *Food Chem Toxicol* 2009; **47**(1): 249–254.
- [11] Ajith TA, Hema U, Aswathy MS. *Zingiber officinale* Roscoe prevents acetaminophen–induced acute hepatotoxicity by enhancing hepatic antioxidant status. *Food Chem Toxicol* 2007; **45**(11): 2267–2272.
- [12] Beutler E. Red cell metabolism. *A manual of biochemical methods*. Third ed. New York: Grune and Startton; 1984.
- [13] Habig WH, Pabst MJ, Jakoby WB. Glutathione S–transferase. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974; **249**(22): 7130–7139.
- [14] Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; **82**(1): 70–77.
- [15] Hwang YP, Choi JH, Jeong HG. Protective effect of the *Aralia continentalis* root extract against carbon tetrachloride–induced hepatotoxicity in mice. *Food Chem Toxicol* 2009; **47**(1): 75–81.
- [16] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurements with the folin phenol reagent. *J Biol Chem* 1951; **193**(1): 265–275.
- [17] Wills PJ, Asha VV. Protective effect of *Lygodium flexuosum* (L.) Sw. extract against carbon tetrachloride–induced acute liver injury in rats. *J Ethnopharmacol* 2006; **108**(3): 320–326.
- [18] Gupta NK, Dixit VK. Hepatoprotective activity of *Cleome viscosa* Linn. extract against thioacetamide–induced hepatotoxicity in rats. *Nat Prod Res* 2009; **23**(14):1289–97
- [19] Bautista M, Andres D, Cascales M, Morales–González JA, Sánchez–Reus MI. Effect of gadolinium chloride on liver regeneration following thioacetamide–induced necrosis in rats. *Int J Mol Sci* 2010; **11**(11): 4426–4440.
- [20] Atef M. Al–Attar. Hepatoprotective Influence of Vitamin C on Thioacetamide–induced liver cirrhosis in Wistar male rats. *J Pharmacol Toxicol* 2011; **6**(3): 218–233.
- [21] Manna P, Sinha M, Pal P, Sil PC. Arjunolic acid, a triterpenoid saponin, ameliorates arsenic–induced cyto–toxicity in hepatocytes. *Chem Biol Interact* 2007; **170**(3): 187–200.
- [22] Jakoby WB. Detoxification, conjugation and hydrolysis in liver biology and pathology. In: Arias IM, Jakoby WB, editors. New York: Raven Press; 1988, p. 375–385.
- [23] Ramanathan K, Balakumar BS, Panneerselvam C. Effects of ascorbic acid and α – tocopherol on arsenic–induced oxidative stress. *Hum Expe Toxicol* 2002; **21**(12): 675–680.
- [24] Celik I, Temur A, Isik I. Hepatoprotective role and antioxidant capacity of pomegranate (*Punica granatum*) flowers infusion against trichloroacetic acid–exposed in rats. *Food Chem Toxicol* 2009; **47**: 145–149.
- [25] Al–Qarawi AA, Mousa HM, Hamed Ali BE, Abdel–Rahman H, El–Mougy SA. Protective effect of extracts from dates (*Phoenix dactylifera* L.) on carbon tetrachloride–induced hepatotoxicity in rats. *Int J Appl Res Vet Med* 2004; **2**(3): 176–180.