International Journal of Phytomedicine 9 (2017) 157-166
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Evaluation of hepatoprotective effect of *Waltheria indica* against various NSAIDs-induced hepatic damage in rats

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Abstract

The objective of the present study was to evaluate methanolic extract of leaves of *Waltheria indica linn.* for hepatoprotective potency of the potent solvent extract. The hepatotoxicity was induced bydiclofenac, carbon tetrachloride (CCl_4)and acetaminophen. In CCl_4 induced hepatotoxicity study, animals were divided into five groups (n=6). Methanolic extract of *Waltheria indica* (WIM) groups were injected in doses of 400 mg/kg and 600mg/kg body weight along with CCl_4 and Silymarin 100mg/kg was taken as standard drug. Similarly procedure was followed in diclofenac and acetaminophen induced hepatotoxicity. Blood samples and liver were collected and liver hisopathological studies were carried out.These histopathological analysis suggested that WIM extract have the ability to reduce the degree of hepatic fibrosis induced by various factors. And concluded that WIM extract has significant hepatoprotective activity thus this study scientifically support the theory to use of this plant in traditional medicine for the treatment of liver disorders.

Keywords: Diclofenac, Carbon tetrachloride (CCl 4) and Acetaminophen, Waltheria indica methanolic extract (WIM), hepatoprotective activity.

Introduction

The liver is a versatile organ which is responsible for the metabolism of chemicals and for the regulation of internal chemical environment. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles. Liver functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Additionally, it also handles the metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them [1].The liver is a major target organ for toxicity of xenobiotics and drugs, because most of the orally ingested chemicals and drugs first go to liver where they are metabolized into toxic intermediates. A large number of xenobiotics are reported to be potentially hepatotoxic [2].

Nearly all of the NSAIDs have been implicated in causing liver injury. Diclofenac, and particularly sulindac, are reported to be more commonly associated with hepatotoxicity, Several NSAIDs have been withdrawn from clinical use because of associated hepatotoxicity [3]. The new more selective COX-2 inhibitors (e.g. celecoxib, rofecoxib, nimesulide) are also associated with hepatotoxicity [4]. Hepatotoxicity from NSAIDs can occur at any time after drug administration, but like most adverse drug reactions, most commonly occurs within 6–12 weeks of initiation of therapy. There are two main clinical patterns of hepatotoxicity due to NSAIDs. The first is an acute hepatitis with jaundice, fever, nausea, greatly elevated transaminases and sometimes eosinophilia.

Material and methods

Hepatoprotective studies

Diclofenac induced hepatotoxicity

Five groups were made with six rats in each. Control Group (Group 1) served as normal and received the vehicle alone (Sterile distilled water, 10 ml/kg, p.o.) for 5 days. Toxin control group (Group 2) animals received diclofenac (50 mg/kg i.p.) on the 3rd and 4th day. WIM-400 (Group 3) and WIM-600 (Group 4) were treated with WIM 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[5]. Standard group (Group 5)was treated for 5 days with standard drug Silymarin (100 mg/kg p.o.) and diclofenac (50 mg/kg i.p.) on the 3rd and 4th day was given 1 h after the treatment of the drug. After 48 hrs animals were sacrificed the last injection of diclofenac under mild ether anesthesia. The blood was collected and allowed to stand for 30 min at 37 C and then centrifuged to separate the serum to estimate various biochemical parameters.

DOI:10.5138/09750185.2081

Carbon tetrachloride induced hepatotoxicity

Five groups were made with six rats in each. Control Group (Group 1) served as normal and received the vehicle alone (Sterile distilled water, 10 ml/kg, p.o.) for 5 days. Toxin control group (Group 2) animals received CCl_4 (2 ml/kg, 1:1 in olive oil, i.p.) [46] on the 3rd and 4th day. WIM-400 (Group 3) and WIM-600 (Group 4) were treated with WIM 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 CCl_4 (2 ml/kg, 1:1 in olive oil, i.p.) was given 1 h after the treatment of the extract. Standard group (Group 5)was treated with standard drug silymarin (100 mg/kg p.o.) for 5 days and on the 3rd and 4th day CCl_4 (2 ml/kg, 1:1 in olive oil, i.p.) was given 1 h after the treatment of the drug. The animals were sacrificed 48 h after the last injection of CCl_4 under mild ether anesthesia. The blood was collected and allowed to stand for 30 min at 37 C and then centrifuged to separate the serum to estimate various biochemical parameters.

Acetaminophen induced hepatotoxicity

Five groups were made with six rats in each.Control Group (Group 1) served as normal and received the vehicle alone (Sterile distilled water, 10 ml/kg, p.o.) for 5 days. Toxin control group (Group 2) animals received acetaminophen (3 g/kg, p.o.) [7] on the 4th day. WIM-400 (Group 3) and WIM-600 (Group 4) were treated with WIM 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 4th day acetaminophen (3 g/kg, p.o.) 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 4th day acetaminophen (3 g/kg, p.o.) was given 1 h after the treatment of the drug. The animals were sacrificed 48 h after the dose of acetaminophen under mild ether anesthesia. The blood was collected and allowed to stand for 30 min at 37 C and then centrifuged to separate the serum to estimate various biochemical parameters.
In above three hepatoprotective models, various in vivo antioxidant parameters were estimated from liver.

Preparation of liver homogenate

Afterlife of rat the liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). Chilled Tris-HCl buffer (0.025 M, pH 7.4) was used as a homogenizer and a portion of the liver was homogenized. The homogenate that was obtained made to centrifuge at 5,000 rpm for 10 min, supernatant was collected and used for analysis.

Biochemical analysis from serum

The biochemical parameters was measured under absorbance spectrophotometry in a UV–VIS Spectrophotometer - 1601 (Shimadzu, Tokyo, Japan).

Estimation of total protein content [8]

The serum total protein was estimated by modified Biuret method (Yatzidis, 1977) using the total protein test kit (Span Diagnostics Ltd.).

Estimation of albumin content [9]

The serum albumin was estimated by the method given by Corcoran and Durnan (1977) using albumin test kit (Span Diagnostics Ltd.).

Estimation of blood urea nitrogen (bun) content [10]

Estimation of serum blood urea nitrogen done by Enzymatic Urease (Berthelot) method, Fawcett and Scott. (1960). using Urea Berthelot test kit (Span Diagnostics Ltd.).

Estimation of alkaline phosphatase (alp) activity

Alkaline phosphatase activity was estimated by the method of Kind and King (1954) using ALP test kit (Span Diagnostics Ltd.).

Estimation of aspartate aminotransferase (ast) activity[11]

The serum aspartate aminotransferase was estimated by the method of, Reitman and Frankel. (1957). using AST test kit (Span Diagnostics Ltd.).

Estimation of alanine aminotransferase (ALT) activity [12]

The serum alanine aminotransferase was estimated by the method of, Reitman and Frankel. (1957). using ALT test kit (Span Diagnostics Ltd.).

Histopathological study [17]

The liver was transferred to 4% formalin solution for fixation and later on processed for histopathological studies following the standard procedure described by Raghuramulu et al. (1983). The microtome sections were cut processed and stained with hematoxylin and eosin. The section thus obtained was scanned in

Carl-Zeiss microscope (Germany) with photographic facility and photomicrographs were taken. Changes if any in the cytoarchitecture were noticed.

Fixation

Fixation is the process of preserving, hardening and preventing postmortem changes of the tissues. The tissues were excised out immediately after sacrificing, cleaned of extraneous matter, cut in to pieces of such thickness that the fixative readily penetrated throughout the tissue to be fixed. Tissue was transferred to the 4% formaldehyde solution and allowed to remain in it till they were taken up for processing.

Tissue processing

Tissue processing involves dehydration, clearing and infiltration of the tissue with paraffin. The usual dehydrating agent is ethyl alcohol; acetone and isopropyl alcohol can also be used. Following dehydration, the tissue was transferred to a paraffin solvent, which is miscible with the dehydrating agent as well. These are known as clearing agents such as chloroform and xylene. Tissue were thoroughly washed by placing them under running tap water and then conveyed through a series of the following solvents as per schedule for dehydration, clearing and paraffin infiltration.

Alcohol 70% - 20 minutes
Alcohol 80% - 20 minutes
Alcohol 90% - 20 minutes
Alcohol 95% (2 changes) - 20 minutes each
Isopropyl alcohol - 20 minutes
Acetone (2 changes) - 20 minutes each
Chloroform (3 changes) - 20 minutes each
Melted paraffin wax (60 C) (3 changes) - 30 minutes each

Then the tissues were embedded in paraffin wax to prepare tissue blocks, which were oriented so that sections could be cut in desired plane of the tissue. Tissues were then fixed to metal object holder after trimming them to suitable size.

Section cutting

A smear of 5% Mayer's egg albumin was prepared and smeared onto the slide and dried. The tissue sections of 6 μm thickness were cut with the help of Spencer type rotating microtome. The tissue sections were put on slide and then section were floated in water on slide at 55-60 C, water drained off and slide dried on hot plate at about 50 C for 30 minutes. This section was ready for staining.

Staining procedure

Reagents

1) Mayer's hematoxylin stain
2) Eosin stain, 2% w/v in alcohol

After fixing the sections on slides, they were stained by serially placing them in the following reagents:

Xylol (2 changes) - 3 minutes
Acetone - 3 minutes
Alcohol 95% - 3 minutes
Haematoxyline stain - 20 minutes
Running water - 20 minutes
Eosin stain - 5 minutes
Alcohol 95% (3 changes) - 3 minutes each
Acetone (2 changes) - 3 minutes each
Xylol (2 changes) - 3 minutes each

After passing through all the above reagents and stains, the slides were mounted with D.P.X. (Diphenyl Phthalate Xylene) and cover slip was placed. Care was taken to avoid air bubbles while mounting the slide.

Results

Hepatoprotective Studies

Diclofenac induced hepatotoxicity

Serum biochemical parameters in pre-treatment of WIM can be determined with respect to induction of hepatotoxicity using diclofenac and the results were shown in Table 1. In the group treated with diclofenac (toxin control), total protein and albumin depleted and were significantly decreased ($P < 0.001$) when compared with the normal control group. In the group treated with diclofenac, the BUN and ALP levels increased significantly ($P < 0.01$, $P < 0.001$ respectively). The administration of diclofenac markedly increased serum AST and ALT levels which were significant as compared to normal control group ($P < 0.05$, $P < 0.01$ respectively).

The groups that received the pre-treatment of WIM 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 ($P < 0.05$) in the serum total protein level as compared to toxin control group. The albumin level in lower as well as in higher dose group increased significantly ($P < 0.01$, $P <0.001$ respectively) as compared to toxin control group and the effect was comparable with the standard group ($P < 0.01$) treated with silymarin. The BUN level decreased in both the dose groups significantly ($P < 0.05$) as compared to toxin control group. The ALP level also significantly decreased in WIM-400 ($P < 0.05$) as well as in WIM- 600 group ($P < 0.001$). In WIM-600 group, the level of ALT and AST significantly decreased ($P < 0.05$), the result was comparable to that of standard group.

Relative liver weight, liver total protein, GSH and antioxidant enzymes in diclofenac induced hepatotoxicity was determined and the results of are given in Table 1. 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 ($P < 0.001$, $P < 0.01$ respectively) in toxin control group. The catalase (CAT) and GPx activity in the toxin control group was also significantly ($P < 0.001$, $P < 0.05$ respectively) depleted as compared to the normal control group. The mean relative liver weight decreased significantly in WIM-400 ($P < 0.001$) and WIM-600.

Table 1: Determination of the effect of methanol extract of *Waltheria indica* leaves on different serum biochemical parameters in diclofenac (50 mg/kg) induced hepatic damage in rats.

Treatment group	Total Protein (g/dl)	Albumin (g/dl)	BUN (mg/dl)	ALP (KA Unit	AST (IU/L)	ALT (IU/L)
Group 1	6.8	4.5	17	23	96	72
Group 2	5.3	2.9	38	47	190	115
Group 3	5.8	3.5	23	36	120	80
Group 4	5.9	3.6	22	22	115	78
Group 5	5.9	3.4	20	22	115	76

Group I: Normal control, Group II: Toxin control diclofenac, Group III: WIM-400 mg/kg + diclofenac, Group IV: WIM-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 < 0.01 as compared with toxin control group.

Table 2: Determination of the effect of methanol extract of *Waltheria indica* leaves on relative liver weight, liver total protein and different liver antioxidants in diclofenac (50 mg/kg) induced hepatic damage in rats.

Treatment group	Liver total Protein (mg/g)	Liver Weight (g/100g b.w.)	GSH (uM/mg protein)	Catalase activity (U/mg)	GPx (U/mg protein)
Group 1	125	2.5	4	170	0.12
Group 2	95	3.8	3	55	0.1
Group 3	105	3.3	4.4	110	0.013
Group 4	108	3.2	4	135	0.1.35
Group 5	110	3.1	4.1	150	0.104

Group I: Normal control, Group II: Toxin control diclofenac, Group III: WIM-400 mg/kg + diclofenac, Group IV: WIM-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 <0.01 as compared with toxin control group.

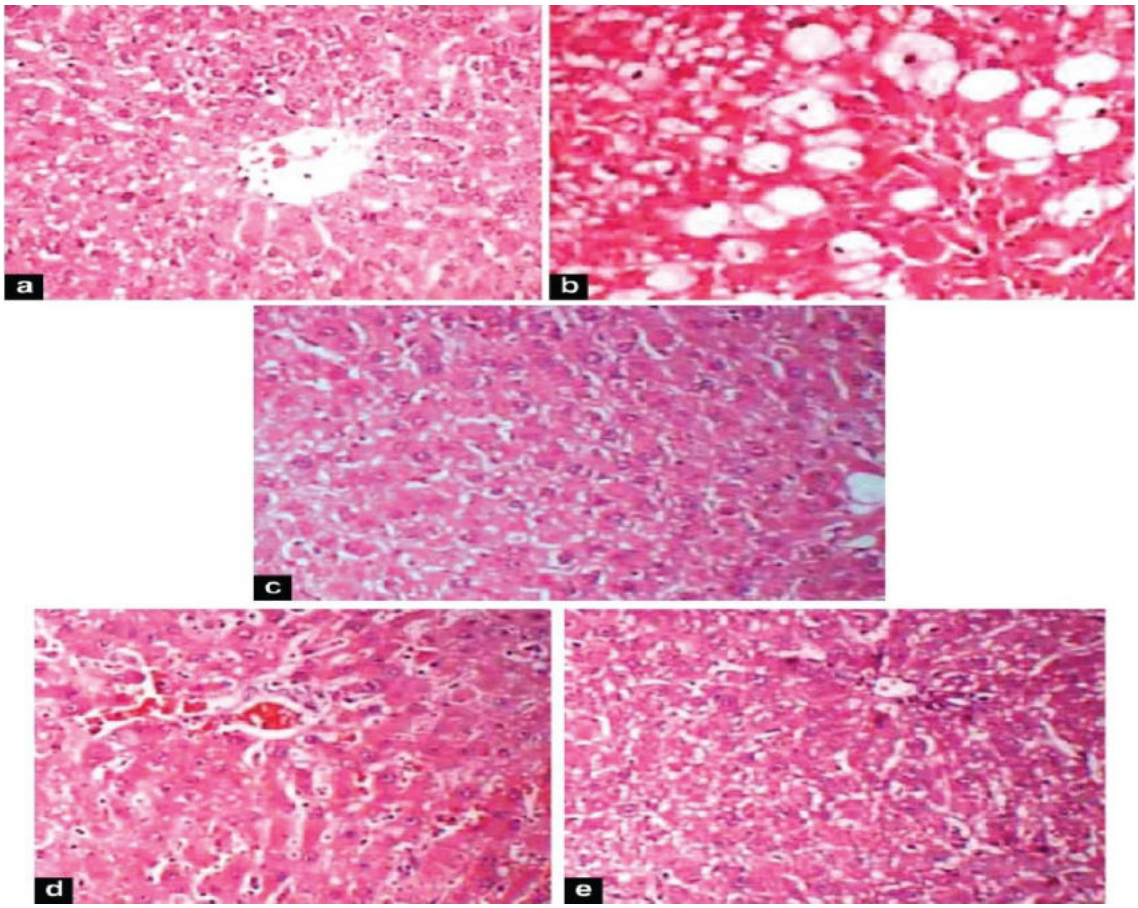

Figure 1: Images of different liver sections of diclofenac (50mg/kg) toxicity in rats(hematoxylinandeosinstained,10x).(a)Normal control group, (b)Toxin control (diclofenac) group,(c)WIM-400mg/kg+diclofenac,(d)WIM-600mg/kg+diclofenac, (e)Silymarin-100mg/kg+diclofenac.

($P < 0.01$) treated group as compared to the toxin control group. The result shows that the higher dose group was comparable to the standard drug treated group ($P < 0.001$). There is elevation in the total protein and GSH levels from liver homogenate in WIM treated groups, but total protein level was not significantly elevated. However, pretreatment with WIM significantly recovered the diclofenac induced GSH depletion in lower and higher dose group ($P < 0.01$, $P < 0.05$ respectively). The catalase and GPx activity increased at both the dose levels; at higher dose WIM exhibited good activity ($P < 0.01$, $P < 0.05$ respectively). GPx activity of WIM-600 group was similar to that of standard drug treated group.

Figure 1A provides information about the normal control animal liver, histological sections showed hepatocytes that were well-preserved, uniform cytoplasm and sinusoidal spaces. Figure 1B gives valuable information about 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 1C & 3D, explains the histopathological hepatic lesions induced by administration of diclofenac was remarkably improved by the treatment with both the doses of WIM and showed marked protective effect by decreasing hepatocellular degeneration and necrosis. Figure 1E gives information about the protective effect which was also observed in silymarin treated animals. This was in good agreement with the results of serum aminotransferase activity and hepatic oxidative stress level.

Carbon tetrachloride induced hepatotoxicity

Table 3 gives the summative results observed from serum biochemical parameters in pre-treatment of WIM with respect to induction of hepatotoxicity using CCl_4. It was observed that there is a marked reduction in total protein and albumin levels was observed in the group treated with CCl_4 and they were significantly decreased ($P < 0.05$) when compared with the normal control group. There is increase in the BUN and ALP levels in the group treated with CCl_4 but not to a significant level. Significant liver damage was observed in the rats treated with CCl_4 (toxin control) and it was commonly indicated by increase in the levels of hepato specific enzymes like AST ($P < 0.01$) and ALT ($P < 0.001$) in serum.

WIM at dose levels of 400 and 600 mg/kg body weight significantly controlled the change in the biochemical parameters as the groups received the pre-treatment of WIM. There is significant increase ($P < 0.01$, $p< 0.05$ respectively) in the serum total protein level by using extract at dose levels of 400 and 600 mg/kg as compared to toxin

control group and the effect was comparable with the standard group ($P < 0.01$) treated with Silymarin (Sily-100). In drug treated groups the albumin level increased but not to a significant level. The level of BUN was reduced in both the dose of WIM and standard drug treated groups, but it was not significant. The ALP ($P < 0.05$), AST ($P< 0.01$) and ALT ($P < 0.01$) levels significantly decreased in WIM-400 group as compared to toxin control group. WIM-600 group also showed significant decreased ($P < 0.05$) AST and ALP levels. The result of relative liver weight, liver total protein, GSH and antioxidant enzymes in CCl_4 induced hepatotoxicity are given in Table 4.

As compared to normal control group, the relative liver weight in toxin control group was significantly increased ($P < 0.001$). The total protein level in liver decreased significantly ($P < 0.001$) in toxin control group. The level of GSH in toxin control group decreased, but it was non- significant. The catalase (CAT) and GPx activities in the toxin control group depleted significantly ($P < 0.05$, $P < 0.001$ respectively) as compared to the normal control group. The mean relative liver weight in WIM at both the doses was slightly elevated as compared to the toxin control group. The total protein level in liver, in WIM treated as well as in the standard drug treated group, increased significantly ($P < 0.001$) as compared to toxin control group. GSH level increased significantly ($P < 0.01$) at higher dose as compared to toxin control group. Catalase activity increased at both the dose levels though not significantly, while in silymarin group, catalase activity decreased. Administration of WIM did not display effect of increase in the GPx activity.

Figure 2A gives the histopathological feature, as shown in indicated the normal liver lobular architecture and cell structure of the liver in the normal control animals. It was observed that there were no pathological changes seen in normal control animals. In CCl_4 treated animals, there was a vacuolar degeneration of hepatocytes around central vein with moderate to severe hepatocyte necrosis due to CCl_4 toxicity (Figure 2B). The histological observations also supported results obtained from the serum enzyme levels.

Table 3: Determination of the influence of methanol extract of *Waltheria indica* leaves on different serum biochemical parameters in CCl_4 (2 ml/kg) induced hepatic damage in rats.

Treatment group	Total Protein (g/dl)	Albumin (g/dl)	BUN (mg/dl)	ALP (KA Unit	AST (IU/L)	ALT (IU/L)
Group 1	6.3	4.2	22	32	105	55
Group 2	5.8	3.5	24	37	150	95
Group 3	6.3	3.5	20	24	104	75
Group 4	6.2	3.5	19	25	120	80
Group 5	6.3	3.7	21	22	105	85

Group I: Normal control, Group II: Toxin control CCl_4, Group III: WFM-400 mg/kg + CCl_4, Group IV: WFM- 600 mg/kg + CCl_4, Group V: Silymarin-100 mg/kg + CCl4. 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 < 0.01 as compared with toxin control group.

Table 4: Determination of influence of methanol extract of *Waltheria indica* leaves on relative liver weight, liver total protein and different liver antioxidants in CCl_4 (2 ml/kg) induced hepatic damage in rats.

Treatment group	Liver total Protein (mg/g)	Liver Weight (g/100g b.w.)	GSH (uM/mg protein)	Catalase activity (U/mg)	GPx (U/mg protein)
Group 1	105	2.7	4.2	170	0.125
Group 2	95	3.2	4.1	145	0.105
Group 3	110	3.3	4.3	155	0.1
Group 4	112	3.4	4.9	150	0.106
Group 5	113	3.3	4.5	130	0.123

Group I: Normal control, Group II: Toxin control CCl4, Group III: WFM-400 mg/kg + CCl4, Group IV: WFM-600 mg/kg + CCl4, Group V: Silymarin-100 mg/kg + CCl4. 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 < 0.01 as compared with toxin control group.

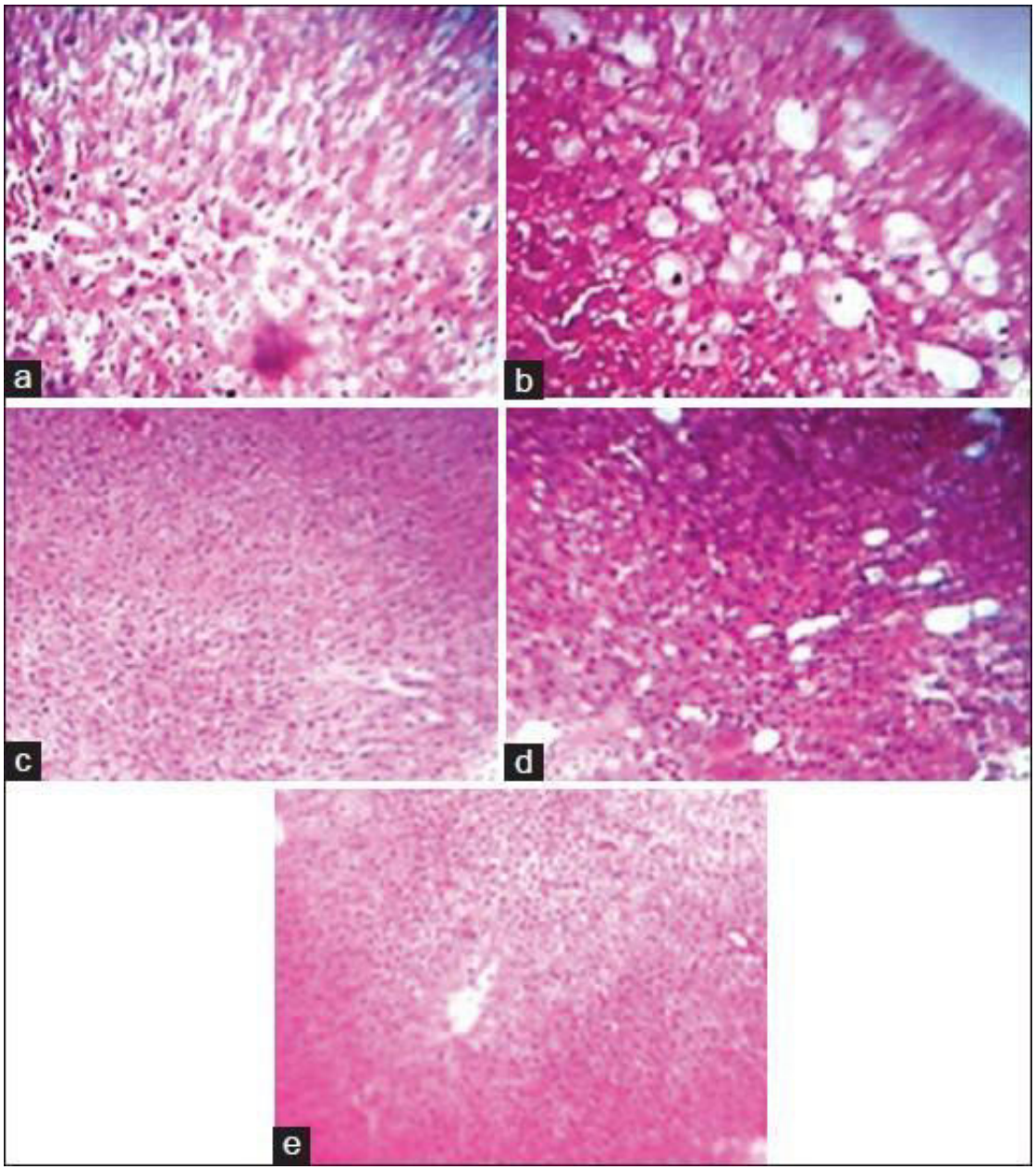

Figure 2: Images for liver sections of CCl_4 (2 ml/kg) toxicity in rats (hematoxylin and eosin stained, 10x). (A) Normal control, (B) Toxin control (CCl_4), (C) WIM-400 + CCl_4, (D) WIM-600 + CCl_4, (E) Silymarin-100 + CCl In case of WIM treated animals, histopathological changes were remarkably reversed, in dose dependently manner with lesser vacuolar degeneration and hepatic necrosis. Similar changes were also observed in the silymarin treated animals.

Acetaminophen induced hepatotoxicity

Table 5 explains oral administration of Acetaminophen (APAP) which is expressed significant liver damage as evidenced by altered biochemical parameters APAP significantly ($P < 0.001$) decreased serum levels of total protein and albumin as compared to normal control group. APAP significantly ($P < 0.01$) enhanced BUN, ALP, AST and ALT levels in the blood circulation; about 3-fold increase was observed in AST and ALT levels in serum.

Treatment with WIM did not exhibit potential effect on recovery of total protein and albumin levels; while in standard drug treated group, the level of total protein and albumin levels increased significantly ($P < 0.01$). The BUN and ALP levels also decreased significantly in lower as well as higher dose of WIM ($P < 0.01$, $P <$

0.05 respectively) as compared to toxin control group. 400 and 600 mg/kg of WIM treated group showed significant ($P < 0.001$, $P < 0.01$ respectively) decrease in AST level as compared to toxin control group. The result of AST was similar to that of the standard drug treated group ($P < 0.001$). ALT level decreased in WIM treated groups towards normalization though not significantly.

The result of relative liver weight, liver total protein, GSH and antioxidant enzymes in APAP induced hepatotoxicity are given in Table 6. The administration of APAP significantly increased the liver weight ($P < 0.001$) as compared to normal control group.

Significant decreased level was observed in hepatic total protein ($P < 0.001$). The administration of APAP significantly decreased the hepatic non-enzymatic antioxidant GSH contents ($P < 0.05$). The treatment of WIM decreased liver weight significantly ($P < 0.05$) at both the dose levels as compared to toxin control group.

Hepatic total protein level in higher dose, increased significantly ($P < 0.01$). WIM-600 in terms of hepatoprotective efficacy was comparable with that of standard drug silymarin. WIM treatment enhanced the production of GSH towards normal control, but not to a significant level. Administration of APAP did not diminish the anti-oxidative status of hepatic catalase and GPx activity.

Table 5: Determination of influence of methanol extract of *Waltheria indica* leaves on different serum biochemical parameters in APAP (3 g/kg) induced hepatic damage in rats.

Treatment group	Total Protein (g/dl)	Albumin (g/dl)	BUN (mg/dl)	ALP (KA Unit	AST (IU/L)	ALT (IU/L)
Group 1	6.8	4.5	17.5	23	100	80
Group 2	6.4	3.5	19.5	61	350	260
Group 3	5.9	3.8	14.6	20	98	120
Group 4	5.8	3.5	14.8	26	145	190
Group 5	6.6	4.05	14.9	17	143	110

Group I: Normal control, Group II: Toxin control APAP, Group III: WIM-400 mg/kg + APAP, Group IV: WIM-600 mg/kg + APAP, Group V: Silymarin-100 mg/kg + APAP. 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 < 0.01 as compared with toxin control group.

Table 6: Determination of influence of methanol extract of *Waltheria indica* leaves on relative liver weight, liver total protein and different liver antioxidants in APAP (3 g/kg) induced hepatic damage in rats.

Treatment group	Liver total Protein (mg/g)	Liver Weight (g/100g b.w.)	GSH (uM/mg protein)	Catalase activity (U/mg)	GPx (U/mg protein)
Group 1	115	2.5	4	165	0.11
Group 2	105	3.5	2.9	220	0.111
Group 3	110	3.2	3.5	225	0.12
Group 4	114	3.25	3.6	205	0.105
Group 5	113	3.1	3.4	215	0.11

Group I: Normal control, Group II: Toxin control APAP, Group III: WFM- 400 mg/kg + APAP, Group IV: WFM-600 mg/kg + APAP, Group V: Silymarin-100 mg/kg + APAP. 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 < 0.01 as compared with toxin control group.

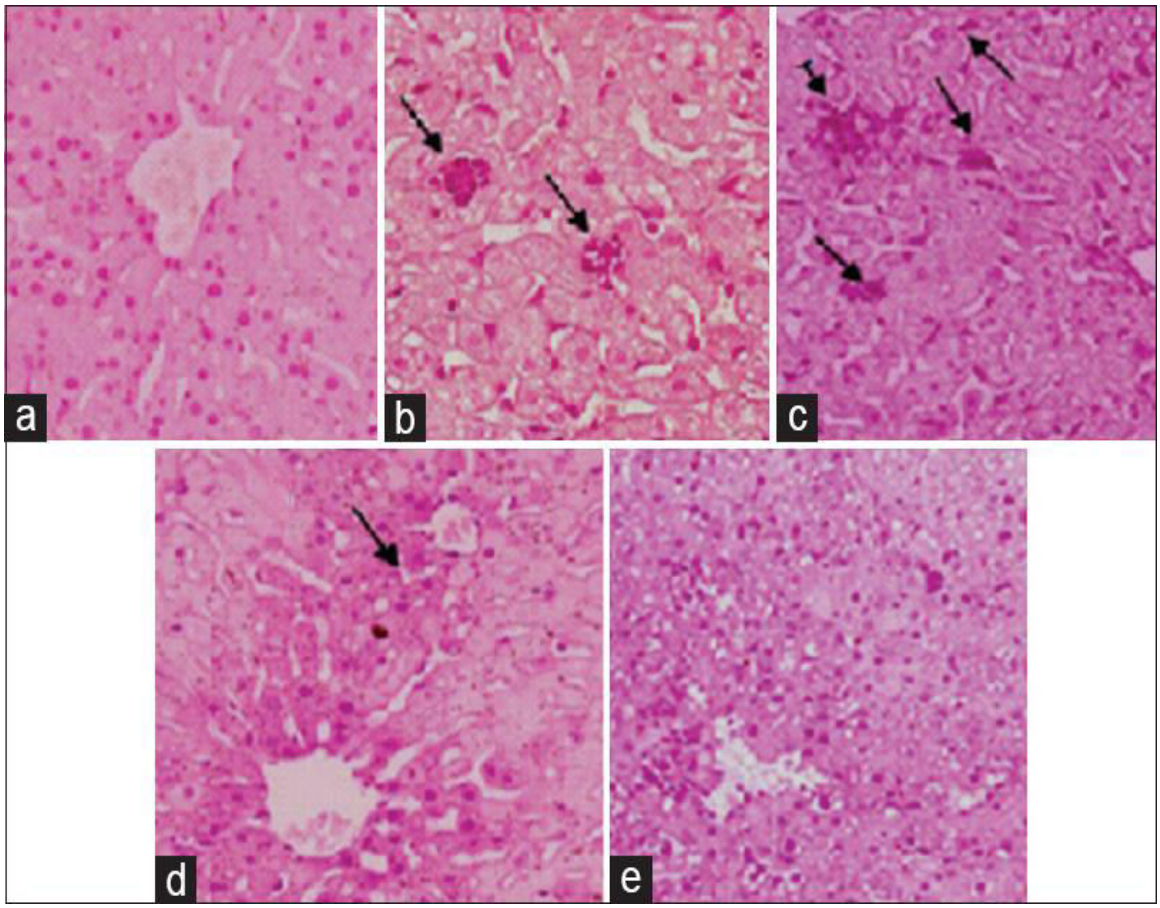

Figure 3: Images of liver sections of APAP (3 g/kg) toxicity in rats (hematoxylin and eosin stained, 10x). (A) Normal control, (B) Toxin control (APAP), (C) WIM-400 + APAP, (D) WIM-600 + APAP, (E) Silymarin-100 + APAP.

Figure 3A explains that no histological abnormalities in normal control liver in Liver histopathologic examination; the hepatic lobular architecture was normal, connective tissue proliferation was not seen. Figure 3B suggests that in acetaminophen treated animals, the liver pathological changes were characterized by severe hepatocellular degeneration and necrosis due to acetaminophen toxicity. In Figure 3C & 5D, WIM protects the liver tissue against acetaminophen toxicity, with mild hepatocellular degeneration, necrosis, less inflammatory cell infiltration and well preserved hepatocytes were observed in most areas when compared with that of APAP group. Figure 3E suggests that recovery from degeneration of hepatic cells of WIM treated animals was comparable to that of standard drug treated animals.

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