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Evaluation of Antioxidant and Hepatoprotective Effect of *Acacia modesta* Wall against Paracetamol Induced Hepatotoxicity

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Authors' contributions

This work was carried out in collaboration between both authors. Author MSR performed the experimental work, managed the literature searches and wrote the initial draft of the manuscript, author MAC designed the protocols of study, supervise the study, wrote the final draft and performed statistical analysis. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: Aim of present study was to investigate the protective effect of *Acacia modesta* bark (Am. Cr) and its proposed mechanism against Paracetamol-induced hepatotoxicity in mice.
Study Design: Albino mice (20-30 g) of either sex were divided into five groups with six animals in each. Group-I was considered as–ve control (received normal saline), Group-II as + ve control (received paracetamol), while Group-III, IV and V as trail (received crud extract of *A. modesta* bark 12.5, 25 and 50 mg/kg respectively). Blood samples were collected by cardiac puncture for analysis of different markers of hepatotoxicity.
Place and Duration of Study: Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan, between April 2013 and 0ctober 2013.
Methodology: Hepatotoxicity was assessed by evaluation of serum levels of hepatic metabolic enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and metabolic content; Bilirubin and level of plasma proteins synthesize by liver (albumin and globulin), by using Randox Test Kits and biochemical analyzer Microlab 300

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respectively. The antioxidant effect of Am.Cr was assayed through in vitro analysis of free radical scavenging activity by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution.
Results: Am. Cr decreases the hepatic enzymes; AST, ALT and ALP, which indicates that Am.Cr contain hepatoprotective constituent(s). Am. Cr bark extract protected against decrease in serum Albumin and Total Protein levels caused by Paracetamol. Am. Cr also showed a good antioxidant activity comparable to Ascorbic acid. Presence of antioxidant constituent(s) was also supported by Tannins and saponins, found during phytochemical analyses of crude extract.
Conclusion: The results indicate the presence of hepatoprotective constituent(s) in extract of *A. modesta* bark and give the logical bases for the use of *A. modesta* as hepatoprotective regime and strongly suggest that this hepatoprotective effect is due to the presence of MDME (microsomal drug metabolizing enzyme) inhibition and antioxidant activity.

Keywords: Acacia modesta; paracetamol; hepatoprotective; DPPH; free radical scavenging.

1. INTRODUCTION

Being the major drug metabolizing and detoxifying organ, liver is subjected to potential damage from enormous pharmaceutical and environmental chemicals [1]. Paracetamol (PCM) a well-known antipyretic and analgesic agent is harmless at therapeutic doses but over dosage can cause life threatening liver damage in experimental animals as well as in humans [2]. Accidental or intentional self-poisoning of PCM is not surprising because it is freely available in pharmacies without prescription and also being kept in many homes [3].

Medicinal plants play a key role in the human health care. About 80% of the world population rely on the use of traditional medicine which is predominantly based on plant materials [4] because, due to increased incidence of adverse effects from the synthetic use of drugs in various ailments. Which can be overcome with the traditionally used medicine found in different regions all over the world that is obtained from plant extracts. A wide variety of researches have been done to investigate several activities of traditionally used plants [5] and now a days medicinal plants are being considered as a potential source of new drugs both in developing countries and the industrialized world [6].

A considerable number of hepatoprotective plants and herbs are known through folklore some of them has be evaluated on scientific basis like; *Amaranthus spinosus, Glycyrrhiza glabra, Momordica dioica, Orthosiphon stamineus* [4]. But still there is a list of hepatoprotective plants, waiting for introduction to modern therapy through pharmacological testing by modern research methods. *Acacia modesta* Wall. (Local name: Phulai) commonly known as Black Sally belongs to family; *Mimosaceae*; a medium sized deciduous tree, grow at waste places in dry soil. Found in Afghanistan, India (Punjab, Uttar Pradesh) and Pakistan (Western Himalayas, Hazara, Peshawar, Rawalpindi, Margalla Hills, Swat, Dir, Salt Range Kashmir and Attock) used as health tonic [7], sexual tonic, restaurant and aphrodisiac. It is effective in stomach disorder [8], leucorrhoea, sexual debility, gleets and back pain. Leaves are used as fodder by goats and sheep. Young twigs are used as miswak (tooth stick) that is effective against dental pathogen [9,10].

Phytochemicals studies indicate that plant contains alpha- amyrin, betulin, octacosanal, e-sitosterol, pet-ether, alcohol and fatty acids [9]. Despite of its medical importance a very few scientific studies are available about it medicinal activities. Different studies indicate the presence of antimicrobial activity and [8], antifungal activity [11] and of hypoglycemic activity [12].

To the best of our knowledge, hepatoprotective effect of *A. modesta* against PCM -induced liver injury in mice has not been demonstrated systematically. Hence, the present study was conducted to evaluate hepatoprotective potential of aqueous-methanolic extract of *A. modesta* (Am. Cr) and its possible underlying mechanisms.

2. EXPERIMENTAL DETAILS

2.1 Plant Material and Extract Preparation

A. modesta stem bark was collected from Soon Valley district Khushab, Pakistan and identified by an expert taxonomist Prof. Dr. Amin Ullah Shah, Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan. The bark was shed dried and powdered finely with

the help of herbal grinder. Aqueous methanol extract of *A. modesta* was prepared by maceration in 80% methanol. The extract was concentrated to a semisolids mass at 40ºC under reduced pressure on a rotary evaporator (BUCHI Labortechnik AG, Switzerland) with an approximate yield of 12%.

2.2 Preliminary Phytochemical Analysis

Am. Cr was subjected to phytochemical analysis for alkaloids, tannins, glycosides, saponin, reducing sugars, anthracene derivative flavonoids as previously followed by ur Rahaman [13].

2.3 Chemicals and Instruments

All drugs and chemicals of analytical grade, used for experimental work were purchased from authentic sources. Methanol, Ethanol and Paracetamol from Merck Chemical Co. Germany, Methyl Cellulose, Ascorbic acid and DPPH from Sigma-Aldrich Co. USA. Enzymatic Test Kits from Randox Laboratories Ltd. London, biochemical analyzer Microlab 300 from Merck & Co., Inc. USA and Shimadzu 1601 UV-Visible spectrophotometer were used for experimental work.

2.4 Experimental Animals

Healthy adult albino mice (20-30 g) of either sex were used for experimental work, housed in metal cages and provided free access to standard feed and tap water. Before starting experiments, mice were acclimatized and observed for 7 days under controlled conditions in the animal house at Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan. Mice were divided into five groups with six animals in each. Group-I and II received 4 doses of 3mL/Kg (body weight) normal saline at 12 hours interval and one hour after the last dose, 1% Methyl cellulose solution was administered in Group-I and stat dose of PCM 640 mg/kg (body weight) was administered to Group-II to induce the hepatotoxicity, by using 1% Methyl cellulose solution as vehicle [14,15].

While the Group-III, IV and V received 4 doses of crud extract of *A. modesta* bark at concentrations 12.5, 25 and 50 mg/Kg respectively, at 12 hours interval. Doses were administered orally by using normal saline as vehicle and one hour after the last dose of extract, PCM 640 mg/Kg was administered to all these groups (Group-III, IV and V). After 24 hour of PCM administration, blood samples were collected by cardiac puncture, centrifuged at 3000 rpm for 15 minutes to separate serum for analysis of different markers of hepatotoxicity [16].

All animals were handled according to the guidelines of Institute of Laboratory Animal Research, [17], approved by the Ethical Committee of The University of Lahore, Lahore, Pakistan.

2.5 Biochemical Analysis

Hepatotoxicity can be assessed by evaluation of serum levels of hepatic metabolic enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST) [18], Alkaline phosphatase (ALP) [19] and level of plasma proteins synthesized by liver (albumin and globulin) [20,21]. Randox Test Kits and biochemical analyzer Microlab 300 were used to evaluate the level of serum ALT, AST, ALP, Albumin and Total protein.

2.6 In-vitro Antioxidant Analysis

The antioxidant effect of Am. Cr was assayed through *in vitro* analysis of free radical scavenging activity by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution (0.45 mM) in absolute ethanol (99%) as previously followed by [3,22]. Extracts (0.5 mL) were added with DPPH (1.0 mL) and allowed to react for half an hour in the dark place. This method involves reaction of antioxidant with a stable free radical DPPH. The free radical scavenging activity of Am. Cr was measured by decrease in the optical absorbance of the ethanol solution of DPPH at a wave length 517 nm that was due to reduction of DPPH concentration by antioxidant and compared with ascorbic acid; an antioxidant vitamin [23,24] by using Shimadzu 1601 UV-Visible spectrophotometer.

2.7 Statistical Analysis

The results were expressed as mean ± SEM. The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by “Turkey-Kramer” Multiple Comparisons Test. The values of $P < 0.05$ $P < 0.01$ and $P < 0.001$ were considered to indicate a significant difference between PCM and the extract treated groups.

3. RESULTS

3.1 Preliminary Phytochemical Analysis

The tests were performed for the presence of phytochemical constituents in the crude aqueous methanolic extract of *A. modesta* (Am. Cr) bark and results are as following (Table 1).

Table 1. Results of preliminary phytochemical screening of crude aqueous methanolic extracts of *A. modesta* (Am. Cr) bark

Test	Am. Cr
Alkaloid	-ve
Tannin	+ve
Glycosides	-ve
Saponin	+ve
Reducing sugars,	-ve
Anthracene	-ve
Flavonoids	-ve

3.2 Effects on Serum ALT and AST Level

A toxic increase in serum ALT and AST level were shown by PCM treated group 1544±301 and 2469±237U/L respectively (Mean ± SEM), significantly higher than the Control group (77±28 and 205.5±47.1) respectively. Am. Cr treated groups didn't show significant reduce in PCM induced ALT level (1086 ± 94 and 1381±421) (Fig. 1a) and AST level (2141±636 and 1596±527) (Fig. 1b) at the dose of 12.5 and 50 mg/kg respectively. Whereas significant protection against PCM induced enzymes level 173±85 and 441±270 was found at 25 mg/kg (Figs. 1a and b).

3.3 Effects on Serum ALP Level

A toxic increase in serum ALP level was shown by PCM treated group, significantly higher 841.5±209.6U/L than the Control group (205.5±47.1). Am. Cr treated groups showed significant protection against PCM induced enzyme level 395.8±25.1, 257.7±26.6 and 348.8±60.7 at the dose of 12.5, 25 mg/kg and 50 mg/kg respectively (Fig. 2).

3.4 Effects on Serum Albumin and Total Protein

The serum albumin and total protein level of PCM treated group were non-significantly lower 4.63±1.1 and 8.62±0.94 g/dL than the Control group (6.1±0.68 and 13.67±3.32) respectively. Am. Cr treated group showed protective effect against decreased serum albumin level 6.33±0.63, 6.02±0.6 and 6.23±0.49 (Fig. 3a).and total protein level 13.78±3.01, 11.27±2.21 and 11.82±2.19 (Fig. 3b) at the dose of 12.5,25 and 50 mg/kg respectively, in PCM hepatic injured mice.

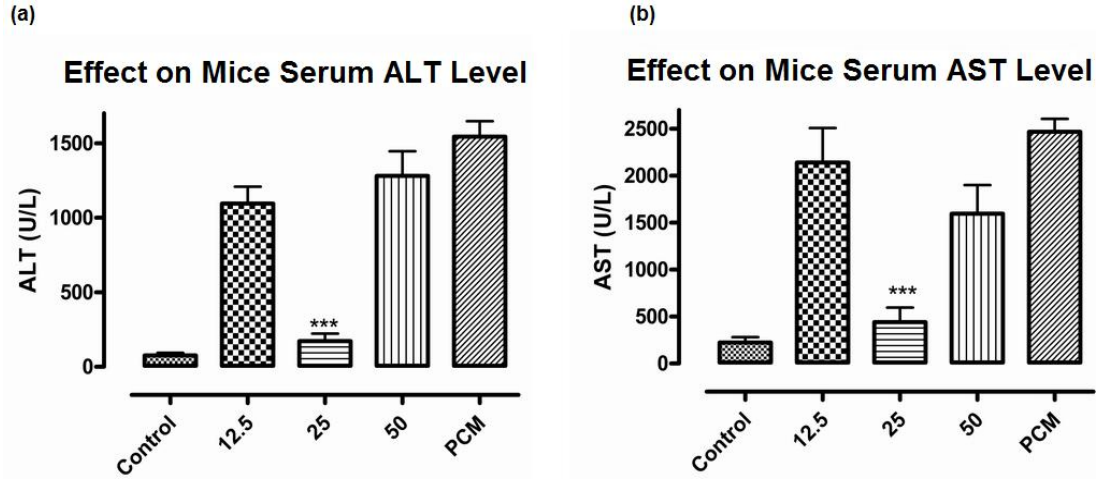

Fig. 1. Bar chart showing the protective effect of crude extract of *A. modesta* bark (Am.Cr) at different concentration (12.5, 25 and 50 mg/Kg of body weight) against PCM (Paracetamol) induced (a) ALT (Alanine Aminotransferase) level and (b) AST (Aspartate Aminotransferase) level

*The responses are shown as concentration of serum ALT and AST in U/L. Values shown are mean ± S.E.M., n =6.(*** P < 0.001 significant difference between PCM and the extract treated group)*

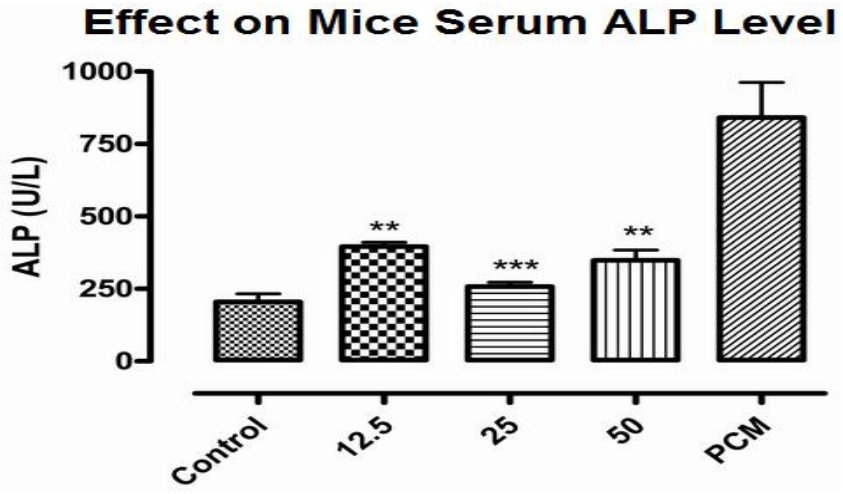

Fig. 2. Bar chart showing the protective effect of crude extract of *A. modesta* bark (Am.Cr) at different concentration (12.5, 25 and 50 mg/Kg of body weight) against PCM (Paracetamol) induced ALP (Alkaline Phosphatase) level

*The responses are shown as concentration of serum ALP in U/L. Values shown are mean ± S.E.M., n =6. (** P < 0.01; *** P 0.001 significant difference between PCM and the extract treated group).*

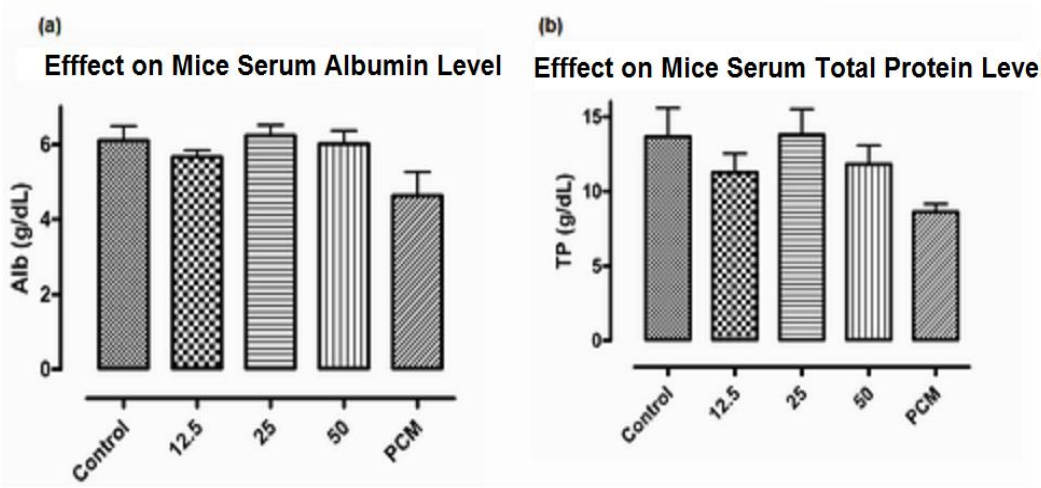

Fig. 3. Bar chart showing the effect of crude extract of *A. modesta* bark (Am.Cr) at different concentration (12.5, 25 and 50 mg/Kg of body weight) against PCM (Paracetamol) induced decreased serum (a) Alb (Albumin) and (b) TP (Total protein) level

The responses are shown as concentration of serum total protein in g/dL. Values shown are mean ± S.E.M., n =6. (No significant difference between PCM and the extract treated group was observed)

3.5 In-vitro Analysis of Antioxidant Effect

Antioxidant effect was measured by free radical scavenging activity. Am. Cr effectively decreased DPPH concentration 76.83±1.1, 83.10±1.2, 84.26±0.8, similar to ascorbic acid 93.85±1.8, 93.35 ± 1.4 and 95.84±1.6 at the concentration of 12.5, 25 and 50 µg/mL respectively (Fig. 4).

4. DISCUSSION

Paracetamol (PCM) is a commonly used analgesic drug. It is safer at normal therapeutic dose but at higher dose it is toxic to liver [25,26]. In PCM-induced hepatotoxicity, serum enzymes such as ALT, AST and ALP levels are elevated [19], whereas the level of plasma proteins (albumin and globulin) synthesized by

liver is decreased, as reported previously [20,21]. But in the present study when administered prophylactically, Am. Cr significantly prevented the PCM induced elevation of ALT, AST and ALP levels and reduction in the plasma proteins level in mice, comparable to control group, indicates the hepatoprotective potential of *A. modesta,* [27].

Since the hepatotoxicity of PCM is related to the production of a highly reactive intermediate metabolite, N-acetyl-p-benzoquinone imine (NAPQI) formed by the cytochrome P_{450} mediated oxidation [19,28]. But under normal conditions NAPQI is readily detoxified by conjugation with hepatic glutathione (GSH) and other detoxifying biomolecules. However at high dose of PCM, excessive production of NAPQI depletes GSH and saturates detoxification pathways, which freely binds to cellular metabolites and cause the hepatic damage [23,29]. So it could be thought that one of the possible hepatoprotective mechanisms of Am. Cr may involve microsomal drug metabolizing enzyme (MDME) inhibition. The inhibition of MDME can reduce the biotransformation of PCM into its reactive metabolite (NAPQI) and hence provide protection against the hepatocellular damage. Moreover oxidative stress caused by excessive generation of NAPQI, damage the lipids and proteins present in biomembranes [16] and the antioxidants compounds can reduce the incidence of oxidative stress, so can effectively prevent liver damage from PCM-induced toxicity [30,31].

Keeping in view all the results, it could be suggested that Am. Cr showed significant protective effect in hepatotoxicity at almost all doses used, but the effects with highest significance ($p < 0.001$) were shown at 25 mg/kg of Am. Cr. Crude extract showed a dose dependent effect at lower doses (12.5 and 25 mg/Kg) and but at higher dose (50 mg/ml), crude extract showed relatively lesser effect that might be due to activation of compensatory mechanism of boy during enzyme inhibition [32].

As Am. Cr extract showed dose dependent strong antioxidant properties that are associated with free radical scavenging activity, comparable to ascorbic acid; an antioxidant vitamin [23,24] that could be beneficial in PCM induced hepatotoxicity. So the other possible hepatoprotective mechanism of Am. Cr extract may also be due to the presence of anti-oxidants in plants as these antioxidants inhibit the covalent binding of NAPQI to vital macromolecules [19].

Based on the phytochemical screening, presence of antioxidant activity in the plant extract is supported by presence of Tannins, because tannins being phenolic compounds are antioxidant in nature [33].

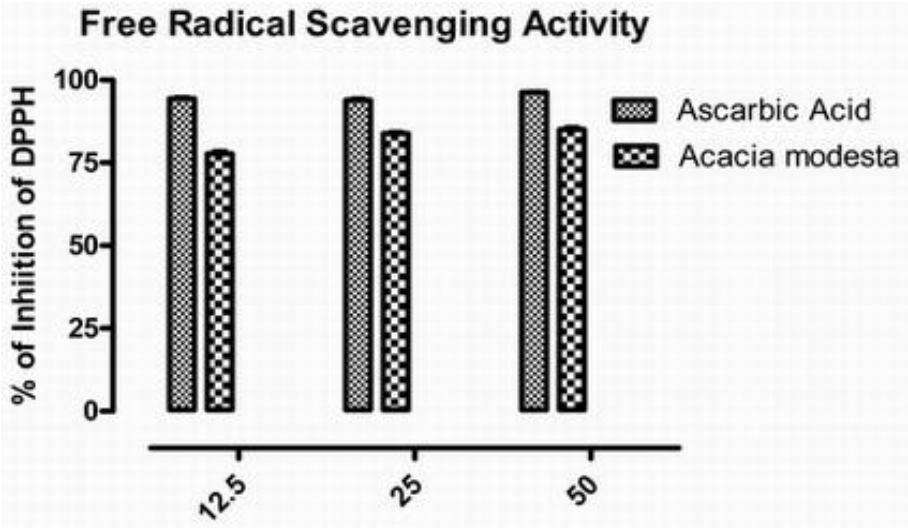

Fig. 4. Bar chart showing the inhibitory effect of Ascorbic acid and crude extract of *A. modesta* bark (Am.Cr) at different concentration (12.5, 25 and 50 µg/mL).

The responses are shown as %age of inhibition of free radical; DPPH (2, 2-diphenyl-1-picrylhydrazyl). Values shown are mean ± S.E.M., n =6.

Since it was a basic study that was done on established laboratory parameters for hepatotoxicity evaluation, further work regarding to *in-vivo* antioxidant activity, isolation and purification of hepatoprotective compound(s) from the crude extract and evaluation with real-time PCR can give the more refined and more specific answer about the hepatoprotective mechanism. Further investigations could lead towards the development of dosage form and be a good addition to health sciences.

5. CONCLUSION

The present investigations give the logical bases for the use of *A. modesta* as hepatoprotective regime and strongly suggest that this hepatoprotective effect is due to the presence of MDME inhibitory and antioxidant activity. So confidently it can be concluded that Am.Cr contained some hepatoprotective biological principle(s).

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CONSENT

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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