HEPATOPROTECTIVE EFFECTS OF AERVA JAVANICA AGAINST PARACETAMOL INDUCED LIVER TOXICITY

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ABSTRACT

Background: Aerva Javanica, a traditional medicinal plant may have hepatoprotective effects. **Objective:** This study was carried out to assess the in-vivo hepatoprotective activity of 70% aqueous methanolic crude extract of Aerva javanica, belonging to the plant family 'Amaranthaceae'. **Methodology:** Wistar Albino rats were divided into six groups, each consisting of six animals. Control and intoxicated groups received normal saline at the dose of 2 ml/kg, standard group received Silymarin (25 mg/kg) and the test groups received three different doses (100, 300 and 1000 mg/kg) of Aj.Cr oral individually for three days. After half an hour of third day treatment, each animal of all the groups, except control, was treated with single per oral dose of 500 mg/kg of Paracetamol (PCM). After 24 hours of intoxication, animals were anesthetized and sera were prepared from blood obtained by cardiac puncture method to assess biochemical parameters; SGOT, SGPT, SALP and total bilirubin (TB), using diagnostic kits. Livers were weighed and samples preserved in 10% formalin for histopathological studies. Acute toxicity studies were also performed in mice. The extract (Aj.Cr) was also analyzed phytochemically for the presence of various secondary metabolites. **Results:** Oral administration of the crude extract of Aerva javanica (Aj.Cr) showed dose-dependent (100-1000 mg/kg) significant reduction ($p<0.05$) in the Paracetamol-induced elevated levels of enzymes; Serum Glutamate Oxaloacetic Transaminase (SGOT), Serum Glutamate Pyruvic Transaminase (SGPT), Serum Alkaline Phosphatase (SALP) and Total Bilirubin (TB) in intoxicated animals. The extract, Aj.Cr, was found to be safe upto the dose of 10 g/kg in mice. Phytochemical analysis showed the presence of several metabolites like glycosides, flavonoids, saponins, terpenes and tannins in the crude extract of the plant. **Conclusion:** The results of the study showed that Aerva Javanica possesses hepatoprotective potential and caused the reversal of abnormal liver functions which justify the traditional use of the plant in liver complaints. However, further investigations are needed to explore the exact mechanism(s) responsible for protective effects of the plant.

Key Words: Aerva javanica, Paracetamol, Hepatotoxicity, SGOT, Total Bilirubin

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INTRODUCTION

Liver plays vital role in the major functions of the body; i.e. metabolism and detoxification and hence is the organ of the body which is maximally exposed to food, drugs, viruses, bacteria, chemicals, poisons, toxins and other xenobiotics.[1] Natural products especially medicinal plants and/or herbs having folkloric and traditional uses have shown important role in the treatment of a number of liver disorders.[2] Plant derived flavonoids,[3] alkaloids,terpenoids,[4] glycosides,[5] saponins,[6] volatile oils, steroids,[7] and tannins,[8] have received considerable attention in recent decades due to their miscellaneous pharmacological properties including antioxidant and hepatoprotective activities. Antioxidants play an important role in providing protection to humans against infectious and degenerative diseases especially by causing inhibition of the production and/or scavenging free radicals.

Aerva javanica (Family: Amaranthaceae), commonly known as Khar Buta, is a perennial herb native to tropical regions of Africa, America and Asian countries (especially, Pakistan and India).[9] The whole plant is used as fodder to the animals and about 169 genera and more than 230 species have been found throughout the world and traditionally used to treat various disorders like diabetes, ulcers, rheumatism, algesia, renal problems, toothache, arthritis,[10] bloody diarrhea, chest pain, edema.[11,12,13] The pharmacological research has revealed the antidiarrheal, antimicrobial,[12] antihyperglycemic, antiplasmodial and cytotoxic activities,[13] of the plant *Aerva javanica*. This study was condcted to assess in vivo hepatoprotective activity of 70% aqueous methanolic crude extract of Aerva Javanica, belonging to plant family Amaranthaceae.

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METHODOLOGY

This experimental study was conducted from 1st March to 1st July 2015 at faculty of Pharmacoogy and alternative medicine, Islamia University Bahawalpur. About 2 kilograms of the leaves of Aerva Javanica were collected after authentication by a botanist, cleaned and dried. A sample of dried leaves was deposited in the herbarium of the Pharmacology Research Lab, Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur, Pakistan; voucher no. AJ-LE-06-14-79 was obtained and kept for future reference. The dried plant material was grounded (National, Japan) to coarse powder and soaked in 70% methanol for 3 days with occasional shaking and filtered first with muslin cloth and then with Whatmann filter paper No. 1. The procedure of soaking and filtration was repeated twice. The combined filtrate was subjected to evaporation under reduced pressure through rotary evaporator (Heidolph, Germany) and a thick viscous paste like crude extract with dark brown color was obtained at the end; i.e. the crude extract (Aj.Cr) with 18.6% yield. Then, it was preserved in light resistant, air tight container with proper labeling and stored in refrigerator at 5°C until use.[14]

Preliminary Phytochemical Analysis

Aj.Cr was subjected to qualitative phytochemical analysis to study the presence of various secondary metabolites; alkaloids, glycosides, tannins, flavonoids, saponins etc. Foam test for Saponins, Ferric chloride test for Tannins, different tests for alkaloids such as Hager's test, Wagner's test, Dragendroff's test and Mayer's test, Keller-Killiani test for glycosides, Alkaline reagent and Lead acetate tests for flavonoids,[15] tests for coumarins, phlobatannins and quinones were performed.[16,17,18]

Experimental Animals

Healthy wistar albino rats (180-250 g) were used for the study after approval by the Animals Research Ethics Committee, department of Pharmacy, faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur (IUB). All the experimental animals were kept in polycarbonated cages of size 47x34x18 cm^3 with standard conditions of temperature (25±2°C) and humidity (50-55%) along with exposure of 12:12 hours light and dark cycle in the animal house of department of Pharmacy, IUB. The standard diet and tap water ad libitum were provided to the animals throughout the study period.

In Vivo Hepatoprotective Activity

The animals were divided into different groups each consisting of six rats. 1st group (control) and 2nd group (intoxicated) received normal saline (Siza International (Pvt) Ltd., Pakistan) at the dose of 2 ml/kg, 3rd group (standard) received 25 mg/kg of silymarin, the reference drug (Abbott Laboratories, Pakistan); whereas, the test groups were administered Aj.Cr at different doses of 100, 300 and 1000 mg/kg of body weight of the animals, oraly once daily for three days. On the 3rd day of treatment, after half an hour of receiving saline, silymarin and Aj.Cr, each animal of all the groups, except control, was administered 500 mg/kg single oral dose of Paracetamol (PCM) that is widely used agent to induce hepatotoxicity in research models. After 24 hours of intoxication, the animals were anaesthetized with the combination of xylazine (10 mg/kg) and ketamine (50 mg/kg) and blood samples were withdrawn by cardiac puncture method. The blood was centrifuged at 2500 rpm for 15 minutes using centrifuge machine (EBA 20 Heltich, Germany) and sera were obtained to assess the levels of different biochemical markers; SGOT, SGPT, total bilirubin TB , and ALP,[18] by using diagnostic kits (Human, Germany). The livers were dissected out from the experimental animals for histopathological study.

Histopathological Studies

The isolated livers were embedded in paraffin, cut into 5 µm thick sections with the help of microtome by serial sectioning and stained with haematoxylin-eosin dye. The slides of liver sections were examined under optical microscope with camera for histological changes; ballooning, degeneration, inflammation, apoptotic cells and fibrosis in liver architecture, loss of nuclei and sinusoid infiltration. Their photomicrographs were also taken.

Toxicity Studies

Acute oral toxicity test was performed by following OECD guidelines. Swiss albino mice (18-25g) were randomly selected to determine mortality and behavioral changes of Aj.Cr in animals. The animals were divided into five groups each consisting of five mice; i.e. group I (control) received normal saline (10 ml/kg) orally. Group II, III, IV and V (test groups) received Aj.Cr at the different doses; 1g/kg, 3g/kg, 10 g/kg and 30 g/kg of body weight, respectively. Different responses; i.e. touch response, pain response, urination, sweating, writhing reflex, hyperactivity, dizziness, convulsions, rightening reflex and fever were observed at 0 minute, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 1

day, 2 days, 1 week and 2 weeks after oral administration of Aj.Cr. The experimental animals received food and tap water *ad libitum* during study period.

Statistical Analysis

The results were analysed statistically and expressed as Mean±S.E.M. One Way Analysis of Variance (ANOVA) with Bonferroni's test was used to compare all pairs of columns using GraphPad Prism Software to observe the significance levels between intoxicated, standard and test groups. P value >0.05 was considered as non-significant (ns), P<0.05 as significant (*), P<0.01 as more significant (**) and P< 0.001 as highly significant (*** or ###).

RESULTS

Preliminary Phytochemical Analysis

The phytochemical analysis of the crude extract of *Aerva javanica* (Aj.Cr) showed the presence of several secondary metabolites; saponins, glycosides, flavonoids, carbohydrates and terpenes; while, rest of the constituents were absent. The results of preliminary phytochemical analysis are given in Table I.

Table I: Preliminary Phytochemical Constituents present in the crude extract of Aerva javanica (Aj.Cr).

Phytochemical Tests	Phytochemical Constituents
Saponins	+
Tannins	+
Alkaloids	-
Glycosides	
Keller-killiani Test	+
Flavonoids	
i) Alkaline Reagent Test	+
ii) Lead Acetate Test	+
Aminoacids and Proteins	
i) Xanthoproteic Test	-
ii) Ninhydrin Test	-
Carbohydrates	
i) Fehling 's Test	-
ii) Benedict's Test	-
iii) Molisch's Test	+
Coumarins	-
Phlobatannins	-
Quinones	-
Phenols	-
Terpenes	
i) Salkowski's Test	+++
ii) Copper Acetate Test	+

(Note: + sign indicates the presence and sign indicates the absence; and number indicates the intensity)

Effects of Aj.Cr on the Levels of SGOT (IU/L) in Paracetamol-intoxicated Albino Rats

The values of SGOT (IU/L) were calculated as 235.4±21.39 in Paracetamol-intoxicated group which were about four to five times higher than those of control group with the values 72.9±3.098. Aj.Cr reduced the levels of SGOT (IU/L) dose-dependently; i.e. 173.6±7.075 at the dose of 100 mg/kg, 136.6±4.375 (p<0.001) at the dose of 300 mg/kg and 84.53±3.197 (p<0.001) at the doe of 1000 mg/kg. The standard group; i.e. Silymarin-treated group showed the values of SGOT of 41.56±7.386 IU/L. (Figure I)

Figure I. The graph showing the dose-dependent effects of the aqueous methanolic crude extract of *Aerva javanica* (Aj.Cr) and the standard drug, Silymarin, on Paracetamol-induced levels of Serum Glutamate Oxaloacetic Transaminase (IU/L). All the values are compared with those of intoxicated group and are considered as non-significant (ns) if p>0.05, significant (*) if p<0.05, more significant (**) if p<0.01, highly significant (***) if p<0.001. The values of intoxicated group are compared with those of control group and considered highly significant (###) if p<0.001.

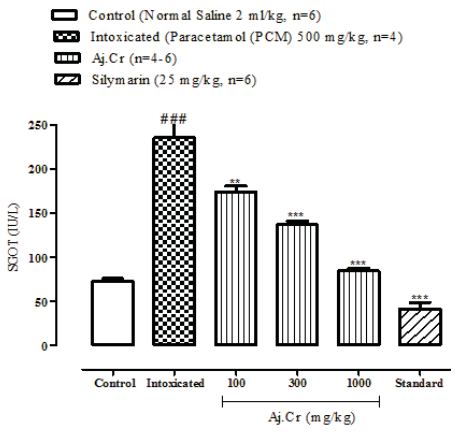
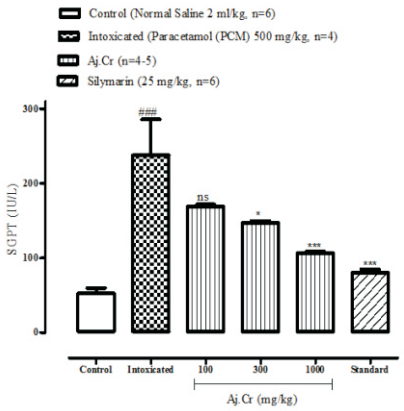


Figure II. The graph showing the dose-dependent effects of the aqueous methanolic crude extract of *Aerva javanica* (Aj.Cr) and Silymarin, the standard hepatoprotective drug, on Paracetamol-induced levels of Serum Glutamate Pyruvate Transaminase (IU/L). All the values are compared with those of intoxicated group and are considered as **non-**significant (ns) if p>0.05, significant (*) if p<0.05 and highly significant (***) if p<0.001. The values of intoxicated group are compared with those of control

group and considered highly significant (###) if p<0.001.



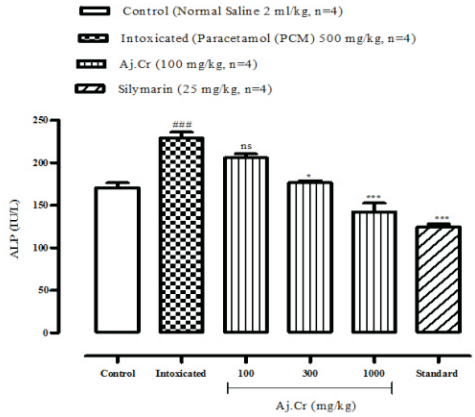
Effects of Aj.Cr on the Levels of SGPT (IU/L) in Paracetamol-intoxicated Albino Rats

The control group showed the levels of SGPT (IU/L) as 52.21±7.499 and standard group 79.89±5.005. The dose-dependent change was seen in reduction of enzyme levels with the values 205.9±4.736, 147.0±2.589 (p<0.05) and 79.89±5.005 (p<0.001) at the doses of 100, 300 and 1000 mg/kg of Aj.Cr, respectively, as compared to the Paracetamol-intoxicated group 235.4±21.39, as shown in Figure II.

Effects of Aj.Cr on the Levels of ALP (IU/L) in Paracetamol-intoxicated Albino Rats

The levels of Alkaline Phosphatase (ALP, IU/L) were noted as 170.7±5.778 in untreated group and 124.5±3.624 in standard group of animals treated with Silymarin. The enzyme levels were elevated in intoxicated group with the values 229.1±6.871 IU/L. Aj.Cr reduced the elevated enzyme levels in dose-dependent manner; i.e. 205.9±4.736 at 100 mg/kg, 176.5±2.389 (p<0.05) at 300 mg/kg and 142.4±10.25 IU/L (p<0.001) at the dose of 1000 mg/kg as shown in Figure III.

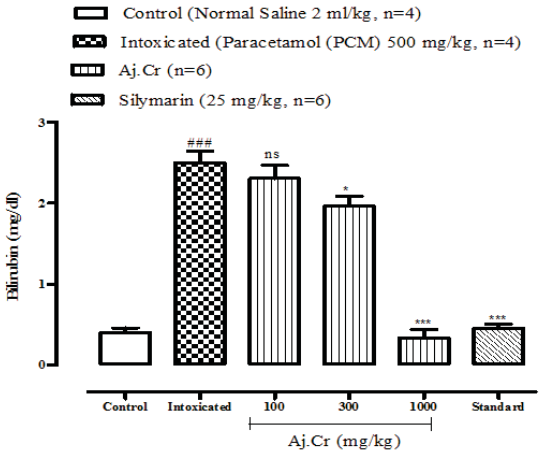
Figure III: The graph showing the dose-dependent effects of the aqueous methanolic crude extract of *Aerva javanica* (Aj.Cr) and Silymarin on Paracetamol-induced levels of Serum Alkaline Phosphatase (IU/L). All the values are compared with those of intoxicated group and are considered as non-significant (ns) if p>0.05, significant (*) if p<0.05 and highly significant (***) if p<0.001. The values of intoxicated group are compared with those of control group and considered highly significant (###) if p<0.001.



Effects of Aj.Cr on the Levels of Total Bilirubin (mg/dl)

Aj.Cr showed reduction in the elevated (2.49±0.14 in Paracetamol-intoxicated group) levels of Total Bilirubin in dose-dependent fashion as 2.30±0.16 at 100 mg/kg, 1.96±0.12 at 300 and 0.33±0.10 at 1000 mg/kg. Silymarin also suppressed the levels of TB upto the normal levels. The details are given in Figure 1V.

Figure IV: The graph showing the dose-dependent effects of the aqueous methanolic crude extract of *Aerva javanica* (Aj.Cr) and the standard drug, Silymarin, on Paracetamol-induced levels of Serum Total Bilirubin (mg/dl). The values are expressed as Mean±S.E.M of 4-6 observations. All the values are compared with those of intoxicated group and are considered as non-significant (ns) if p>0.05, significant (*) if p<0.05 and highly significant (***) if p<0.001. The values of intoxicated group are compared with those of control group and considered highly significant (###) if p<0.001.

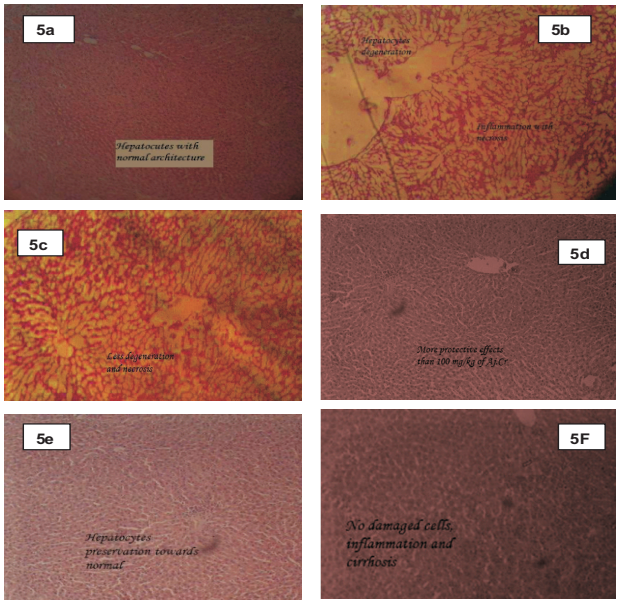


Histopathological Studies

Pretreated animals with the standard drug, Silymarin (25 mg/kg), Aj.Cr at three different doses; 100, 300

and 1000 mg/kg of body weight decreased the degeneration of hepatocytes, extensive ballooning, sinusoid infiltration, inflammation and complete necrosis (Fig.5c, 5d, 5e, and 5f) as compared to hepatotoxins (Paracetamol-acetaminophen/CCl_4), shown in Fig. 5b.

Figure V: Figure showing micrographic (100X) results of slides of liver sections of different groups: (5a) the control group, (5b) extensive ballooning, inflammation, sinusoid infiltration, necrosis and highly degeneration of hepatocytes in Paracetamol-intoxicated group, (5c, 5d and 5e) the dose-dependent effects of Aj.Cr; 100, 300 and 1000 mg/kg, respectively, (5f) protective effects on liver tissue cells exerted by the standard drug, silymarin (25 mg/kg).



Acute Toxicity Study

Aj.Cr was found to be safe upto 10 g/kg of body weight of mice with no behavioral changes observed for 14 days. Mortality was seen at the dose of 30 g/kg which showed the toxicity of the crude extract of Aerva javanica (Aj.Cr) at very high dose.

DISCUSSION

The presence of flavonoids and saponins have been found to exhibit antioxidant as well as hepatoprotective activities.[13] They have also shown the potential to prevent lipid peroxidation by entrapping reactive oxygen radicals.[19] Aj.Cr reduced the levels of SGOT more significantly with $p<0.01$ at the dose of 100 mg/kg and highly significantly with $p<0.001$ at the doses of 300 and 1000 mg/kg as compared to the intoxicated group. The highly elevated levels of enzyme, serum glutamate oxaloacetic transaminase, in Paracetamol (PCM)-intoxicated group were due to the high dose of paracetamol (i.e. 500 mg/kg); while, it is safe at therapeutic doses. PCM is metabolized through liver and is converted to different metabolites. At high doses, it metabolizes into toxic metabolites; N-acetyl-p-benzoquinone-imine (NAPQI) by hepatic isozymes, cytochrome P-450. NAPQI binds to proteins and DNA and forms adducts that cause the dysfunction and death of hepatocytes leading to necrosis; in addition, nephrotoxicity and death of living beings,[20] is also reported. Serum glutamic oxaloacetic transaminase (SGOT) enzyme is the indicator of hepatic functions and the most sensitive as well as specific test and the highly elevated levels indicate the acute and chronic liver disease as well as cardiac necrosis.[21] The observed elevated levels of SGPT indicate the existence of toxic hepatosis, metal poisoning and muscular dystrophy. In the cases of hepatic damage with hepatocellular lesions and parenchymal necrosis, these biochemical enzymes are leaked from the damaged tissues into the blood stream.[22] ALP is considered as an important enzyme that presents the appropriate functioning of liver. Elevated levels of ALP indicate the stability of bilirubin dysfunction during hepatic injury which may be due to drugs or chemicals. Serum bilirubin level is also a common indicator of hepatic function. Serum level of bilirubin is a common indicator of hepatic functions and elevated levels may indicate jaundice and injury of the hepatic cells,[23] Moreover, histopathological effects confirmed the reservation of histo-architecture towards normal, following dose-dependent fashion might indicate the metabolite's protective effects.

CONCLUSION

It is concluded that Aerva javanica, exhibited protective effects on the hepatic tissues against paracetamol-induced hepatotoxicity in rats also contains several secondary metabolites; saponins, flavonoids, terpenes etc. that may be responsible for causing defense against hepatotoxicity and possess antioxidant potential so are hepatoprotective. Hence, the study justifies the traditional uses of the plant against various hepatic disorders. However, further studies are required to be carried out to isolate the purified compound(s) and to explore the possible mechanism(s) responsible for the hepatoprotective potential of the plant.

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