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Hepatoprotective effects of methanolic extract of *Alcea rosea* against acetaminophen-induced hepatotoxicity in mice

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Abstract

The aim of this study was to evaluate the hepatoprotective effects of *Alcea rosea* against acetaminophen-induced hepatotoxicity in mice. Aqueous methanolic extract of *A. rosea* were given orally for 7 consecutive days followed by daily toxic dose of acetaminophen. At the end of treatment period, evaluation of hepatoprotective activity of *A. rosea* was done on basis of levels of liver enzyme markers (aminotransferases, alkaline phosphatase and bilirubin) and histopathological examination of liver tissues. Acetaminophen significantly increased serum levels of liver enzyme markers whereas, the extract of *A. rosea* significantly reduced serum levels of elevated liver enzyme markers in dose-dependent manner compared to acetaminophen treated mice group. Histopathological examination of liver tissues also supported the protective effects of *A. rosea* on liver enzyme markers. We conclude that extract of *A. rosea* has strong hepatoprotective effects against acetaminophen-induced hepatotoxicity; thereby, affirming its traditional therapeutic role in liver injury.

Introduction

Treatment of common liver diseases such as cirrhosis, fatty liver and chronic hepatitis is quite complicated. Available treatments or medications due to their inefficiency and profound adverse effects posed on the liver make them inappropriate to treat liver disorders. This has overall led the reliance on safe and effective medicine of plant source. Studies have shown that the plants like *Carica papaya* (Sadeque and Begum, 2010), *Carissa spinarum* (Hegde and Joshi, 2010), *Cestrum nocturnum* (Qadir et al., 2014), *Chenopodium murale* (Saleem et al., 2014), *Cocculus hirsutus* (Thakare et al., 2009), *Convolvulus arvensis* (Ali et al., 2013), *Dodonaea viscosa* (Khan et al., 2013), *Khamira Gaozaban Ambri Jadwar Ood Saleeb Wala* (Akhtar et al., 2013), *Malva sylvestris*

(Hussain et al., 2014), *Oflpomoea staphylylina* (Bag and Mumtaz, 2013), *Rumex dentatus* (Saleem et al., 2014), *Suaeda fruticosa* (Rehman et al., 2013), *Thymus linearis* (Alamgeer et al, 2014), *Trianthema decandra* (Balamurugan and Muthusamy, 2008), *Trichodesma sedgwickianum* (Saboo et al., 2013) and *Viola odorata* (Qadir et al., 2014) showed hepatoprotective effect.

Alcea rosea (*A. rosea*) commonly known as Hollyhock, is a common ornamental plant in Mallow family, Malvaceae, with large showy blossoms of different shades. It is believed to have originated in China or from the tropical areas of South-East Asia. In herbal medicine, it is used for the treatment of inflammation of the kidneys and the uterus, intestinal tract infections with vomiting and diarrhea, kidney and urinary tract



infections, jaundice, malaria, rheumatism and snake bite (Duke and Ayensu, 1985; Munir et al., 2012). The reported scientific effects of *A. rosea* are anti-microbial (Mert et al., 2010), hypoglycemic activity in diabetic mice (Kulkarni et al., 2002), immunomodulatory property (Ghaoui et al., 2008), analgesic and anti-inflammatory activity (Mert et al., 2010; Wang et al., 1989). Roots of *A. rosea* have also been used for the treatment of inflammation, diarrhea, constipation, bronchitis, severe coughs and angina (Ahmadi et al., 2012). The dry extracts of *A. rosea* stems and roots contain monosaccharides, oligosaccharides, mucus, microelements and proteins. The aim of this study was to investigate the possible hepatoprotective activity of *A. rosea* extract against the hepatotoxicity induced by acetaminophen overdose in mice used as an experimental animal model.

Materials and Methods

Collection and authentication of plant

Aerial parts of *A. rosea* were collected from the fields in Faisalabad, Pakistan during July 2012. The plant was verified and given the voucher No: 617-2-13 at University of Agriculture Faisalabad, Pakistan. The voucher was kept in University of Agriculture Faisalabad for future reference.

Preparation of plant extract

The aerial parts of *A. rosea* were washed by tap water and then air dried. The dried plant was pulverized into coarse powder by mechanical grinder and stored in air tight container. Dried powder (1 kg) was soaked in aqueous methanol (30:70) for seven days with daily shaking. After soaking for seven days, the extract was separated by filtration and concentrated using rotary evaporator at 70°C. The crude extract was stored in air tight container.

Experimental animals

Swiss albino mice, weighing between 20-30 g, were used for the study. The animals were procured from animal house of University of Agriculture Faisalabad, Pakistan and were kept at animal house in College of Pharmacy, Government College University, Faisalabad, Pakistan. After randomization into various groups, the mice were acclimatized for period of 7 days under standard husbandry conditions at room temperature (25 ± 3°C) and 12 hours light/dark cycle. All the animals were fed under strict hygienic conditions with rodent pellet diet and water *ad libitum*.

Experimental design

In this experimental protocol, Swiss albino mice were

selected and divided into five groups of five animals in each group. The test substances were administered by oral gavage using a stomach tube. Prior to dosing, animals were kept for 12 hours of fasting. After giving the dose, food was withheld for further 3-4 hours.

The experimental protocol was designed for seven days. Group A was maintained as control group and received 1 mL/kg normal saline daily. Group B received acetaminophen 250 mg/kg orally for seven consecutive days. Group C animals were treated with silymarin (50 mg/kg, p.o) which served as standard group. Groups D and E animals were treated with two different doses of aqueous methanolic extract of *A. rosea* (200 mg/kg and 400 mg/kg) respectively. Group C, D and E were also intoxicated with acetaminophen (250 mg/kg) 3 hours after the administration of silymarin and extracts of *A. rosea* for seven days.

Biochemical investigation

After 24 hours following the last dose, all the animals were anesthetized with chloroform and sacrificed. The blood was collected in eppendorf tubes and allowed to clot and serum was separated with the help of centrifuge at 4,000 rpm for 20 min. Biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, direct and indirect bilirubin were measured using commercially available kits (Merck, and DiaSys Diagnostic systems GmbH, Germany) according to the standard protocol.

Histopathological studies

Histopathological analysis was performed as described previously (Hussain et al., 2014). All the mice were killed mercifully and their liver tissues were dissected out, washed with ice-cold normal saline. A small cross section of the liver was separated out and embedded in paraffin after fixing with 10% neutral-buffered formalin. These tissues were dehydrated with graded ethanol and cleaned by xylene following paraffin infiltration. Finally, tissue sections were cut in size of 4-5 µm, deparaffinised with xylene and rehydrated with graded isopropyl alcohol and a drop of water. Water was removed and slides were oven dried. After tissue fixation, staining was done with hematoxylin and eosin. The stained sections of slides were examined under high-resolution microscope by blind observer and photographs were taken.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test using GraphPad Prism 5 (GraphPad, Software Inc., USA). The value of significant difference was considered at $p < 0.05$.

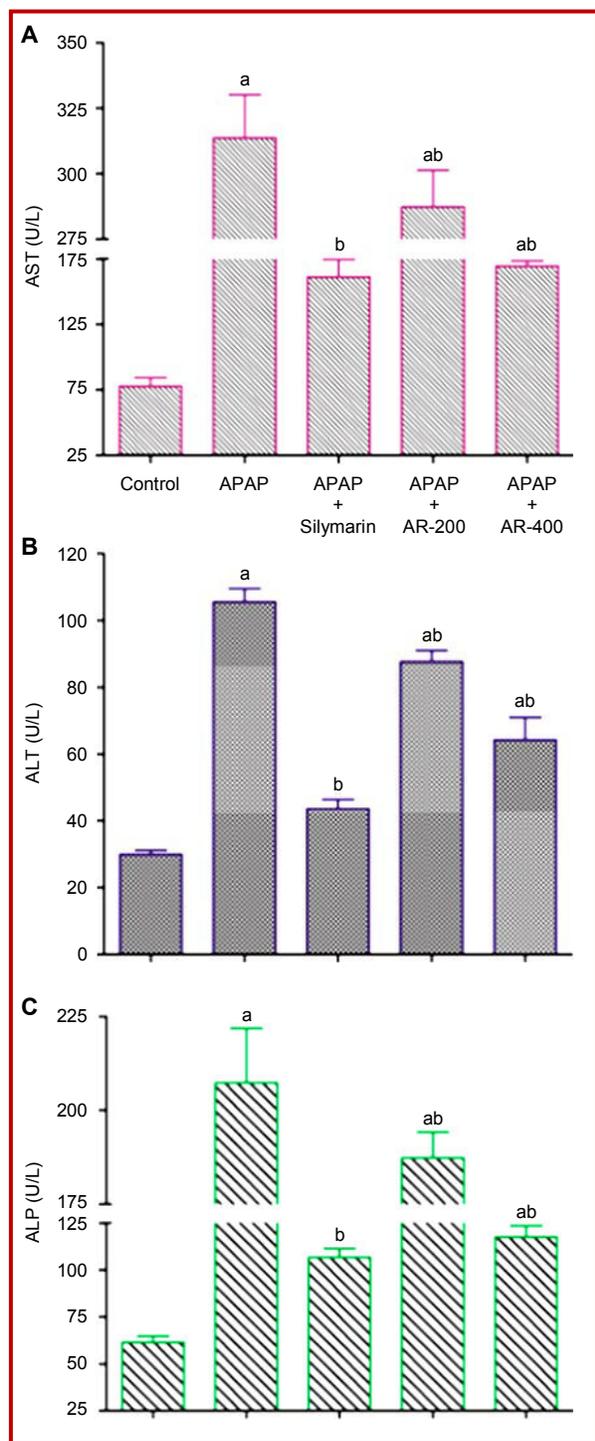


Figure 1: Effect of the methanolic extract of *A. rosea* on liver enzyme markers. AST (A), ALT (B) and ALP (C). AR-200; 200 mg/kg of *A. rosea*, AR-400; 400 mg/kg of *A. rosea*. (* $p < 0.05$) denote the value of experimental groups significantly different from control and (^b $p < 0.05$) denote the value of experimental groups significantly different from the group treated with acetaminophen. APAP; Acetaminophen, AST; aspartate amino transferase, ALT; alanine amino transferase, ALP; alkaline phosphatase

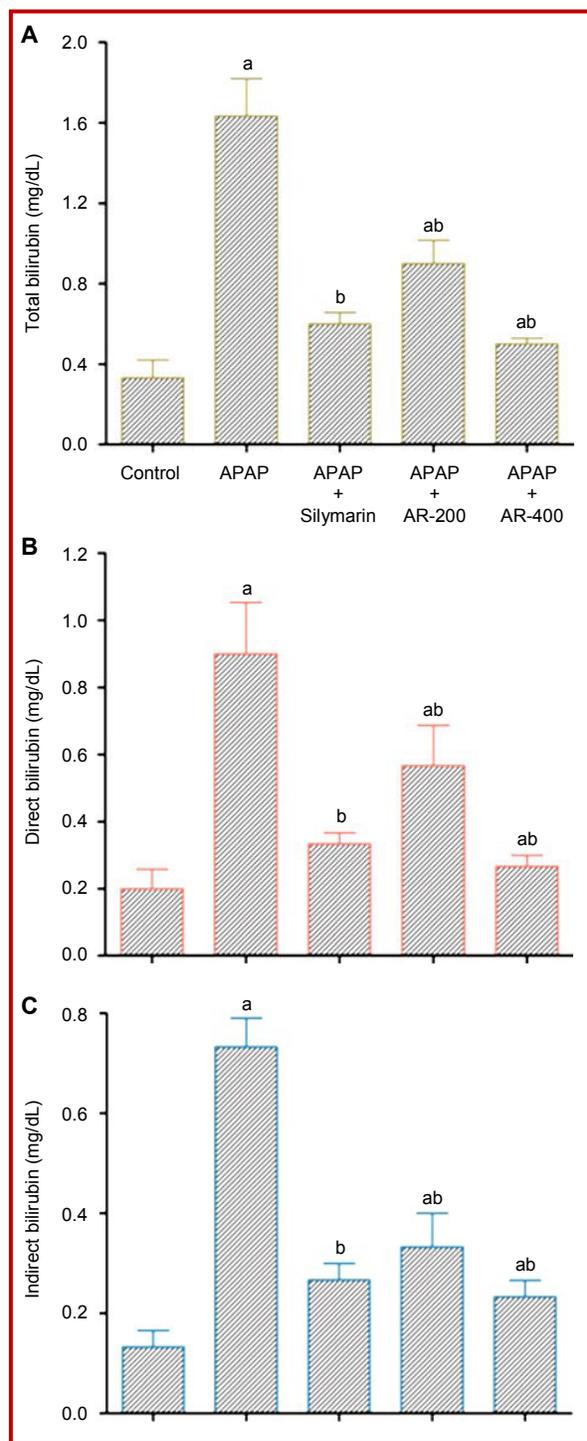


Figure 2: Effect of the methanolic extract of *A. rosea* on bilirubin. Total bilirubin (A), direct bilirubin (B) and indirect bilirubin (C). APAP; Acetaminophen, AR-200; 200 mg/kg of *A. rosea*, AR-400; 400 mg/kg of *A. rosea*. (* $p < 0.05$) denote the value of experimental groups significantly different from control and (^b $p < 0.05$) denote the value of experimental groups significantly different from the group treated with acetaminophen

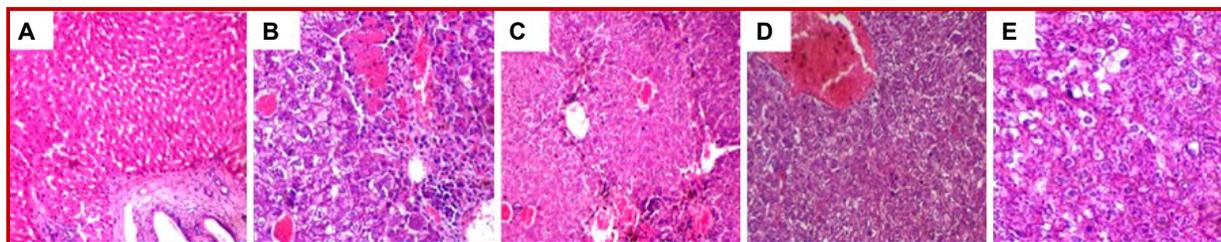


Figure 3: Histopathological examination of liver sections. Photomicrograph of liver from mice administered with normal saline (A), APAP only (B), APAP + Silymarin (C), APAP + AR-200 (D) and APAP + AR-400 (E). APAP; Acetaminophen, AR-200; 200 mg/Kg of *A. rosea*, AR-400; 400 mg/kg of *A. rosea*. All figures are $\times 100$ magnification

Results

Acetaminophen produced a higher concentration of AST up to 313.6 ± 16.4 U/L ($p < 0.01$) in Group B animals compared to the levels in Group A animals (77.6 ± 6.4 U/L) (Figure 1). *A. rosea* at the dose of 200 mg/kg showed no significant effect on the level of AST (287.3 ± 13.9 U/L) in Group D animals as non-significant difference ($p > 0.05$) was observed when directly compared with the levels in Group B animals. Administration of methanolic extract of *A. rosea* at the dose of 400 mg/kg significantly ($p < 0.01$) decreased the AST level in Group E when directly compared to the levels in Group B proving its hepatoprotective nature. *A. rosea* at the dose of 400 mg/kg reduced the AST level (176.6 ± 3.8 U/L) more significantly in Group E and its results were more comparable to the AST levels (161.3 ± 17.0 U/L) in Group C animals as compared to AST levels obtained at 200 mg/kg dose of *A. rosea* in Group D animals.

In Group A animals, ALT level was found to be 30.0 ± 1.1 U/L, which showed that liver was functioning properly but this level was highly increased (105.6 ± 3.9 U/L) in Group B ($p < 0.01$) indicating a decline in hepatic function leading to liver damage (Figure 1). Silymarin decreased the level of ALT (43.6 ± 2.7 U/L) in Group C animals nearly to the levels in Group A animals. The methanolic extract of *A. rosea* at the dose of 400 mg/kg also decreased the ALT levels (64.3 ± 6.6 U/L) that were comparable to the levels of Group A and C animals. Ostensibly, the results obtained from *A. rosea* with the dose of 200 mg/kg showed only a slight decrease of ALT levels (87.6 ± 3.3 U/L) in comparison to the levels of ALT in Group B.

Similarly, ALP levels in Group A animals were 61.3 ± 3.5 U/L. These levels were highly increased in Group B (207.3 ± 14.5 U/L) (Figure 1). Group D showed non-significant ($p > 0.05$) change in ALP levels compared to ALP levels in Group B. *A. rosea* at dose 400 mg/kg showed that plant extract significantly decreased the levels of ALP (120.6 ± 5.9 U/L) in serum same as observed in Group C animals treated with standard drug silymarin (106.6 ± 4.6 U/L). *A. rosea* administered at the dose of 200 mg/kg has not shown any satisfying results giving less or no hepatoprotective activity against acetaminophen as it did not declines ALP

levels. *A. rosea* showed dose-dependent activity at the dose of 200 mg/kg showing less or no hepatoprotective activity and at dose of 400 mg/kg showing stupendous results equivalent to the results obtained in Group C animals.

Acetaminophen, in part of its toxic effects, leads to the fulminant liver damage which can be seen by the elevated levels of bilirubin. The levels of total bilirubin were highly increased in Group B animals (1.6 ± 0.1 mg/dL) treated with acetaminophen than in Group A animals (0.3 ± 0.1 mg/dL) (Figure 2). Groups C, D and E pretreated with silymarin, AR-200 and AR-400 respectively showed improved liver function by lowering the serum levels of total bilirubin compared to acetaminophen-intoxicated animals in Group B. Silymarin showed its standard activity by decreasing the levels of total bilirubin up to 0.6 ± 0.1 mg/dL that were comparable to the levels in Group A. The tested plant Groups D and E displayed the mean values of total bilirubin 0.9 ± 0.1 mg/dL and 0.5 ± 0.1 mg/dL, respectively showing hepatoprotective activity in dose-dependent manner.

Toxic dose of acetaminophen in Group B animals led to the remarkable increase in serum levels of direct bilirubin (0.9 ± 0.1 mg/dL) and indirect bilirubin (0.7 ± 0.1 mg/dL) indicating the liver damage. While in Group A animals, the mean values of direct and indirect bilirubin were 0.2 ± 0.0 mg/dL and 0.1 ± 0.0 mg/dL respectively. Evidently, silymarin displayed the values of direct and indirect bilirubin 0.3 ± 0.0 mg/dL and 0.2 ± 0.0 mg/dL, respectively in Group C that were more or less comparable to Group A. *A. rosea* decreased the levels of direct bilirubin from 0.5 ± 0.1 mg/dL to 0.2 ± 0.0 mg/dL and indirect bilirubin from 0.3 ± 0.0 mg/dL to 0.2 ± 0.0 mg/dL in dose-dependent manner.

Figure 3 shows the histopathological examinations of liver tissues after the treatment period. The liver tissue Group A exhibiting all the hepatocytes arranged in cord fashion with eosinophilic cytoplasm and prominent nucleus containing 1-2 nucleoli. Portal areas showed normal lining of bile duct and hepatic artery. The tissue section intoxicated with acetaminophen in Group B animals. Photomicrograph reveals that the hepatocytes were swollen and lost their structural integrity. It

showed degenerative (cytoplasmic vacuolation) and necrotic changes in hepatocytes. Capillaries were engorged with RBCs (Congestion). The liver section of Group C animals treated with standard drug silymarin. Photomicrograph showed hepatocytes in normal shape and size with normal portal area. There are mild to moderate degenerative and congestive changes. Regenerative activity is maximum. The liver tissues of Group D treated with *A. rosea* with the dose of 200 mg/kg. Hepatocytes showed mild to moderate cytoplasmic vacuolation. Nucleus is vesicular (prominent nucleus). Liver sections from mice treated with *A. rosea* with the dose of 400 mg/kg. Regenerative activity is seen with normal hepatocytes with prominent nucleus. Mild congestion is seen but cytoplasm is clear (no vacuolation). The histological examination proved the protective role *A. rosea* against acetaminophen toxicity.

Discussion

A substantial number of herbal formulations have been affirmed to have medicinal properties to cure several life-threatening diseases (Sabir and Rocha, 2008; Akash et al., 2011; Akash et al., 2014a,b; Bhaskar and Balakrishnan, 2010; Huang et al., 2010; Venkatesh et al., 2010; Nayak et al., 2011; Rehman et al., 2012; Ibrahim et al., 2013; Hussain et al., 2014; Parveen et al., 2014). The plants that exhibit hepatoprotective effects, have the phytoconstituents such as phenyl compounds, coumarins, essential oils, monoterpenoids, diterpenoids, triterpenoids, steroids, alkaloids and other nitrogenous compounds (Sharma et al., 2011; Valan et al., 2010) that reveal hepatoprotective effects by preventing the liver from the damaging effects of drug-induced intoxication (Rehman et al., 2014).

The plant *A. rosea* of family Malvaceae used for this research is traditionally used for a number of remedies. *A. rosea* has been used in homeopathy for the treatment of jaundice. In the present study, *A. rosea* was tested to validate its use as hepatoprotective agent. The extract of *A. rosea*, depicted its hepatoprotective effect maintaining the low levels of liver enzyme markers. Thus it gives a plausible explanation that this plant showed hepatoprotective activity attributed to its phytochemical constituents.

Conclusion

A. rosea exhibits hepatoprotective effects in mice intoxicated with acetaminophen.

Ethical Issue

The experiments on animals were performed in accordance to

the guidelines of Ethical committee Government College University, Faisalabad and were approved by Advanced Studies and Research Board.

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