doi.org/10.36721/PJPS.2023.36.4.REG.1227-1232.1

Hepatoprotective potential of *Anagallis arvensis* (L.) extract against carbon tetrachloride (CCl_4)-induced hepatic injury and oxidative stress in rabbits

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Abstract: *Anagallis arvensis* L. has several health benefits, such as it is an effective remedy for epileptic disorders, leprosy, rheumatism, and hepatic and renal dysfunctions. However, scientific evidence of the plant against liver disease is not reported so far. Thus, the aim of the present study was to highlight the hepatoprotective and hepatocurative effect of extract on hepatic injury induced by carbon tetrachloride (CCl_4). The extract was investigated for its effect on hematological parameters, liver enzymes regulation, and anti-oxidant markers (SOD & CAT). In addition, histopathological investigations were performed. This extract displayed significant reversion of WBCs, RBCs, platelets count, hemoglobin, ALT, AST, ALP and albumin levels towards the normal level as compared with control. Therefore, there was significant rise in level of SOD and CAT in both groups (hepatocurative and protective). Furthermore, histological investigation demonstrated the preventive effect. The presence of alkaloids, carbohydrates, protein, phenolic compounds, tannins and saponins in the extract was confirmed by the preliminary phytochemical studies. Thus, based on all these facts, it can be concluded that *Anagallis arvensis* extract has restorative capability against CCl_4-induced hepatotoxicity and could be used as the hepatocurative and hepatoprotective agent, which could be attributed to the reported secondary metabolites.

Keywords: *Anagallis arvensis* L., hepatoprotective potential, carbon tetrachloride-induced toxicity, oxidative stress.

INTRODUCTION

Liver is one of the most important and relatively large body organs involved in biotransformation and detoxification of the food constituents, xenobiotic and drugs absorbed through the intestine. The purpose of liver function test is to keep an eye on how effectively the liver is performing its duties and the test comprises of various blood tests. The liver enzymes including alanine aminotransferase (ALT), alkaline phosphatases (ALP) and aspartate aminotransferase (AST) are indicators of liver damage (Lala *et al*., 2021).

Current allopathic treatment of liver damage comprises antiviral medications and radiation, surgery and/or chemotherapy. However, the currently available conventional treatments for hepatic disorders have limited efficacy with the serious side effects (Fried, 2002). Currently, scientists are pointing towards traditional medicines to discover new or novel alternative therapeutics for the hepatic disorders as cost-effective with minimal side effects as well as easily available (Rehman *et al*., 2018).

Anagallis arvensis L. also termed as a small scarlet pimpernel (Billi Booti) is the member of the family Primulaceae (Umair *et al*., 2017). It is widely distributed in the regions of Asia, Europe, and north America (Al-Snafi, 2015). Leaf decoction or infusion of *Anagallis arvensis* L. has been traditionally claimed for a wide range of the medicinal use, including for wound healing undefined skin disorders, sinusitis, cough, bronchitis, pneumonia, sore throat, infections, haemorrhoids, hepatic dysfunsctions, thick blood and high blood pressure management across south west Europe (Menendez-Baceta *et al*., 2014).

Moreover, to the best of our knowledge, no scientific evidence is present that shows the hepatoprotective and hepatocurative action of *Anagallis arvensis* L. Therefore, purpose of the present work was to evaluate the *in-vivo* hepatoprotective potential of ethanolic extract of *Anagallis arvensis* L. by performing different hematological and histopathological evaluations.

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MATERIALS AND METHODS

Collection and drying of plant material

Aerial part of *A. arvensis* was purchased from a local herbal practitioner's shop in Hafizabad, Punjab, Pakistan and was washed properly. The plant was identified by Muhammad Naeem Qaiser, University of Sargodha with assigned voucher number of SU/115/19. The plant materials were then dried in open air until it becomes moisture free and grounded using a mechanical grinder. Afterwards, 1000 g of the powdered plant was macerated for 7 days with 5.5L of 95% methanol and filtered through the vacuum suction pump. The solvent was separated from the filtrate using a rotary evaporator at 125 rpm and 37˚C. A sticky oily mass that had semi-solid consistency was separated from the mixture (Shah *et al.,* 2018).

Experiment animals and housing conditions

Healthy albino rabbits of either sex, weighing between 1.5-2 kg were purchased from the local market of Lahore. The animals were housed in the animal house of University of Sargodha under hygienic and well ventilated conditions at 23-25˚C under natural light and dark cycle. Animals were feed with standard laboratory diet and water *ad libitum* (Vipin *et al.,* 2017).

Preliminary phytochemical screening

Preliminary screenings of phytochemical constituents were performed for the determination of alkaloids, carbohydrates, protein, phenolic compounds, tannins and saponins by using the standard procedures with minor modifications. Wagner reagent was used to detect alkaloids, whereas, Molish and Benedict test were used to confirm the presence of carbohydrates. Biuret test was used to evaluate protein presence in the extracts. Lead acetate and ferric chloride test were used to determine the presence of phenolic compounds and tannins, respectively. Moreover, vigorous shaking of plant extracts in water was done to indicate the presence of saponins (Banu and Cathrine, 2015). Furthermore, spot test and Legal test were performed to identify presence of fixed oil or fat and glycoside, respectively.

Induction of the hepatic injury

Administration of carbon tetrachloride (CCl_4) subcutaneously at a dose of 1.25mL/kg suspended in olive oil in a 1:1 ratio induced the hepatic injury in the hepatocurative model (Sahreen *et al.,* 2011).

Hepatoprotective and hepatocurative study

Hepatocurative investigation of plant extracts was done according to study conducted by Bukhsh *et al.* (2014) with some amendments. In this model, albino rabbits were divided into four groups containing five rabbits (n=5) in each group in a random fashion with the following specifications:

Group A (normal control), received the vehicle olive oil. Group B (disease/sham control): CCl_4 (1.25 mL/kg, SC). Group C: This group served as hepatocurative. Animals of this group were injected CCl_4 at the start of experiments and then treated with the oral administration of *Anagallis arvensis* L. (4 mg/kg) with olive oil in 1:1 for 30 days. All rabbits were sacrificed at the end of experiment to collect blood samples for hematological studies and dissect liver for histopathology studies. Then these blood samples were analyzed for determination of liver enzymes. Group D: This group served as hepatoprotective and was consisted of the rabbits that were directly treated with oral administration of *Anagallis arvensis* L. (4 mg/kg) with olive oil in 1:1 for 29 days followed by one dose of carbon tetrachloride (CCl_4) (1.25 mL/kg), 2 h after the last drug dose. Toxicity studies reported toxic effects of this plant almost at dose of 10 mg/kg body weight. Therefore, the dose selected on base of pilot study for above mentioned experiment was 4 mg/kg body weight orally (Al-Sultan *et al.,* 2003).

Hematological evaluation and liver enzymes regulation

The red blood cells (RBCs), white blood cells (WBCs), platelets (PLT) and hemoglobin (Hb) level were measured by a chemical analyzer. The collected blood samples were centrifuged immediately to separate the serum at a speed of 4000 rpm for 5 min. The hepatic damage was assessed by visualizing the changes of the serum markers level of the ALT, ALP, AST and albumin. They were analyzed by using commercially available kits following the manufacturer's instructions and the results were expressed in the units of international units per liter (IU/L) (Saleem *et al*., 2018).

Evaluation of superoxide dismutase (SOD) and catalase enzyme (CAT)

Estimation of SOD and CAT was done according to kit manufacturer protocols in liver of all groups (Adeyemi and Olayaki, 2018).

Histopathological evaluation

The stored sections of the liver were fixed in formalin and thin sections (4µm thick liver sections) were prepared, stained with hematoxylin & eosin (H&E) dye following the previously established protocols. These sections were then evaluated histologically under a light microscope equipped with a camera (Rehman *et al.,* 2018).

STATISTICAL ANALYSIS

The results were statistically computed using GraphPad Prism® (version 6.0) and One-way analysis of variance (ANOVA) followed by the Tukey's post hoc tests were employed to perform multiple comparisons between and within groups. Value ($p<0.005$) was considered as significant.

Table 1: Hepatocurative and hepatoprotective effect of plant extract

Groups	Liver anti-oxidant enzymes	
	SOD	CAT
Group A (Normal control)	7.61±0.260	5.28±0.28
Group B (disease control)	4.34±0.055	4.0±0.04
Group C (hepato-curative)	8.1±0.24****	8.1±0.09****
Group D (hepato-protective)	7.73±0.39****	7.30±0.12****

Results were expressed in the form of Mean±SEM.

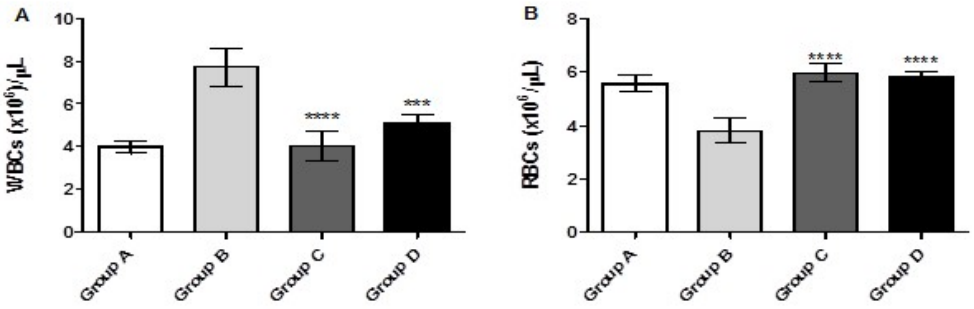

Fig. 1: Effects of oral application of *Anagallis arvensis* L. extracts (4 mg/kg/body weight) followed by CCl_4 administration in rabbits (Group D) on [A] white blood cells (WBCs) and [B] red blood cells (RBCs) compared with control (Group B serving as toxic control), CCl_4-given rabbits (Group B) and hepatocurative group (Group C). Values are means ± SD (n = 5), (***P<0.001), ****P<0.0001).

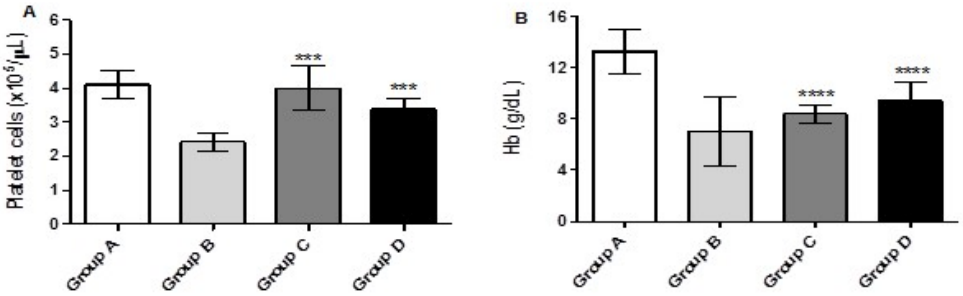

Fig. 2: Effects of oral application of *Anagallis arvensis* L. extracts (4 mg/kg/body weight) followed by CCl_4 administration in rabbits (Group D) on [A] platelets and [B] hemoglobin (Hb) compared with control (Group B serving as toxic control), CCl_4-treated rabbits (Group B) and hepatocurative group (Group C). Values are means ± SD (n = 5), (***P<0.001), ****P<0.0001).

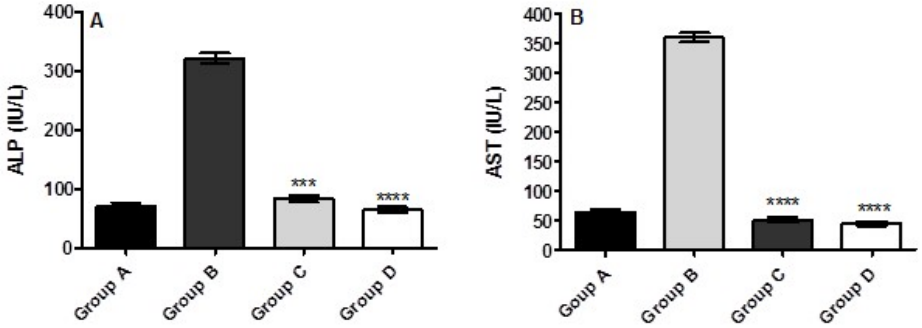

Fig. 3: Effects of oral application of *Anagallis arvensis* L. extracts (4 mg/kg/body weight) followed by CCl_4 administration in rabbits (Group D) on [A] ALP and [B] AST level (IU/L) compared with control (Group B serving as toxic control), CCl_4-treated rabbits (Group B) and hepatocurative group (Group C). Values are means ± SD (n=5), (***P<0.001), ****P<0.0001).

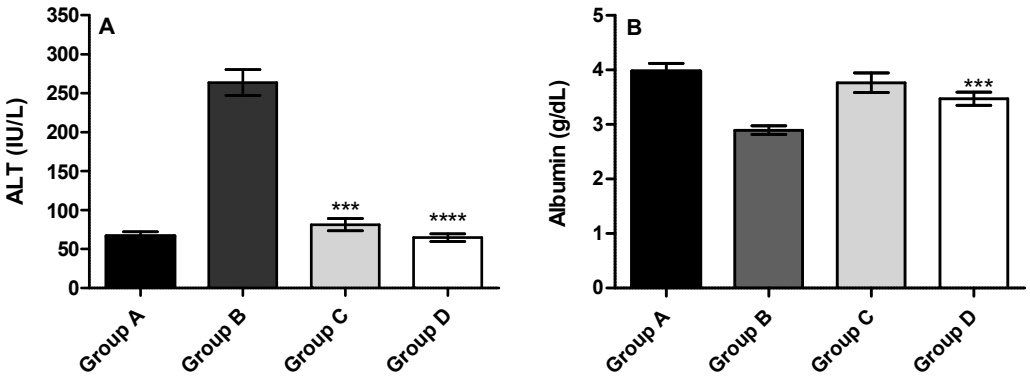

Fig. 4: Effects of oral application of *Anagallis arvensis* L. extracts (4 mg/kg/body weight) followed by CCl_4 administration in rabbits (Group D) on [A] ALT (IU/L) and [B] albumin (g/dL) level (IU/L) compared with control (Group B serving as toxic control), CCl_4-treated rabbits (Group B) and hepatocurative group (Group C). Values are means ± SD (n = 5), (***P<0.001),****P<0.0001).

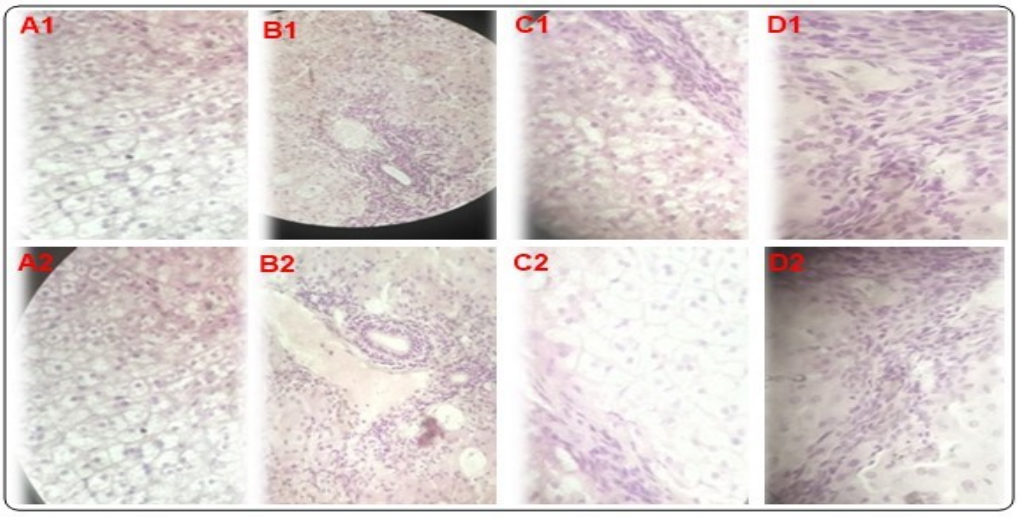

Fig. 5: Staining of rabbit liver tissue (H&E) [A1 and A2] control group (Group A) with normal histological liver appearance showing bright shiny surface with soft and smooth in touching, [B1 and B2] CCl_4-induced liver (Group B) seemed dull and lack the regular shine of the healthy liver due to less blood flow through the livers, [C1 and C2] hepatocurative group (Group C) with relatively healthier as compared to the group B and has bright color and shiny surface [D1 and D2] hepatoprotective group (Group D) seem to be healthy like control.

Fig 6: **Phytochemical profile.** Left one showed negative result of legal test for glycosides as no pink appeared. Middle one showed the presence of alkaloids by brownish ppt. via wagner test. Right one first test tube showed presence of foam, second and third test tubes showed presence of phenolic and tannin compounds via ferric chloride (dark green colour) and lead acetate (bulky white ppt.) respectively.

RESULTS

Phytochemical profile

A. arvensis extract showed positive results for the tests for alkaloids, carbohydrates, protein, phenolic compounds, tannins and saponins as shown in fig. 6. Alkaloid presence was indicated by the brownish precipitate, carbohydrates by violet ring and proteins by pink colour.

Hematological evaluation

The effect of *Anagallis arvensis* L. extracts on different blood cell counts and Hb level is presented in fig. 1 and fig. 2. In the toxic control (group B), the level of WBCs significantly increased whereas this effect was restored by treatment with the herbal extract in Group C and Group D. Similarly, the levels of RBCs in intoxicated animals were increased in treatment group. Moreover, *Anagallis arvensis* L. exhibited significant enhancement on platelets and hemoglobin level as compared with the control.

Liver enzymes regulation

The results of liver damage are depicted in fig. 3 and fig. 4. These enzymatic (ALT, AST, ALP) values were significantly higher ($p<0.001$) in the Group B in comparison Group A. The animals of the group C were cured and group B were relatively protected as their levels were close to the control group. In the hepatocurative group (Group C), the mean values for ALT and albumin were 81±7.7 (IU/L) and 3.7±0.2 (g/dL), respectively which were significantly close to the normal control group. Moreover, the mean values for ALT and albumin of the hepatoprotective group (group D) were 68±6.05 (IU/L) and 3.4±0.12 (g/dL), respectively.

Measurement of SOD and CAT

The results presented in table 1 depicted that there was significant decrease in level of SOD and CAT in disease control group. Whereas, the values of SOD and CAT were significantly ($P<0.0001$) higher in animals treated with extract.

Histopathological evaluation

The microscopic histopathological evaluation of the liver with various treatment groups is presented in fig. 5. The liver of normal control group showed bright shiny surface and soft and smooth in touching (figs. A1 and A2). Group B seemed dull and lack the regular shine of the healthy liver. The rough appearance and faint red color of livers clearly depicted improper and less blood flow through the livers (fig. B1 and B2). Conversely, the liver tissue of the animals in group C were relatively healthier as compared to group B. They had bright color and shiny surface. Moreover, they were comparatively smoother to touch in comparison with the group B (figs. C1 and C2). The liver tissue of animals of hepatoprotective group (group D) seems healthy. Thus, the plant extract had demonstrated a preventive effect. The livers of the hepatocurative group and the hepatoprotective group showed normal hepatocytes with the liver cells infiltration. Fat deposition was visible at some areas. No marked difference in the histology of the treated group as compared to the normal control was observed.

DISCUSSION

The value of any drug is based on the fact that how effectively it restores the normal physiology by eliminating the agents responsible for its pathology. The efficacy of the hepatoprotective and hepatocurative drugs is also based on the same principle of restoring the normal hepatic physiological functions. The study herein represented that *Anagallis arvensis* L. has hepatocurative and hepatoprotective properties. It is evident from the figs.1-4 that it restores normal cell count and decreases the rising enzyme levels of ALT, AST, ALP and serum albumin levels. Groups treated with the extracts depicted considerable protection against the hepatic injury. This study results are in accordance with the previous studies that showed the hepatoprotective effects on the liver injury by induction of the CCl_4 (Eidi *et al*., 2012, Jeong *et al*., 2005, Tirkey *et al*., 2005). The oxidative stress is induced by the carbon tetrachloride which may evoke the hepatic damage by the release of the cytokines, like TGF-β. It could excite the hepatic stellate cells (HSC) which may be responsible to differentiate in myofibroblast like cells. The effect of plant extract on SOD and CAT as mentioned in table 1 showed that the protective effect on liver fibrosis. These results are also in line with the previous studies (Khan *et al*., 2012, Rehman *et al*., 2018, Saleem *et al*., 2018). Phenolic compounds might have outstanding radical scavenging features. Plants with the phenolic compounds have been seen in the previous studies to have a role in the hepatoprotection and the anti-inflammatory activities. The phytochemical profile is evident that alkaloids having anti-inflammatory property and flavonoids and alkaloids having the radical scavenging properties contribute in the hepatocuration and hepatoprotection of liver cells (Hiraganahalli *et al*., 2012).

At the earlier stage, the hepatic injury due to the carbon tetrachloride toxicity may in the noticeable fat droplets in the parenchyma of liver. It has been evident from histological studies that the extract of the *Anagallis arvensis* L. slowed the liver injury process before and after CCl_4 intoxication. The extract treatment might have slowed the process of hepatocytes disruption and increases the speed of hepatic regeneration. Overall, it is evident from the histological perspectives that extract treatments (Group C and D) reinstate the liver injury values close to the normal control (Rehman *et al*., 2018). All these studies suggest that *Anagallis arvensis* L. possesses hepatocurative and the hepatoprotective effects against the carbon tetrachloride (CCl_4) induced hepatic damage.

CONCLUSION

This study shows that *Anagallis arvensis* L. being the natural drug may be used for the protection and curing of the liver injury. There is still need to evaluate the effect of extract on anti-oxidant enzymes.

ACKNOWLEDGEMENT

Authors express appreciation to the Deanship of Scientific research at King Khalid University, Saudi Arabia for funding through research groups program under grant No.R.G. P 1/92/43.

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