

Evaluation of Hepatoprotective Activity of Aqueous Extract of *Andrographis paniculata* in Wistar Rats

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ABSTRACT

Background: The objective of the current study was to evaluate the hepatoprotective activity of the aqueous extract of *Andrographis paniculata* in wistar rats. **Materials and Methods:** The animals were divided into six groups with six rats in each group. First group was taken as control, and received 0.9% normal saline 10 ml/kg body weight orally. Second group was taken as CCl₄ control group and treated with normal saline (10 ml/ kg, p.o.) and CCl₄: olive oil (1:1, 2 ml/ kg, i.p. on 2, 5 and 8th day) daily for 20 day. The third group rats was treated with Liv 52- 100 mg/kg orally and CCl₄: olive oil (1:1, 2 ml/ kg, i.p. on 2, 5 and 8th day) for 20 days. The fourth and fifth groups were taken as test groups and they received crude extract of *Andrographis paniculata* orally once daily for 20 days at the dosage of 250 and 500 mg/kg respectively. CCl₄: olive oil was given i.p on 2, 5 and 8th day. On the 21st day, blood samples were collected by cardiac puncture method. The blood samples were used to estimate biochemical parameters like serum SGOT, SGPT, ALT, bilirubin and cholesterol and the liver was preserved for histopathological examination. **Results:** It was found that the *Andrographis paniculata* exhibited moderate protective effect by lowering the serum levels of SGOT, SGPT, ALT, bilirubin and cholesterol (P<0.001). Histology of liver section of animal treated with the extracts showed the hepatic cell regeneration. **Conclusion:** *Andrographis paniculata* has dose dependant hepatoprotective activity.

Key words: Carbon Tetrachloride, Hepatoprotective Activity, *Andrographis paniculata*, Liver Disease, Herbals.

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INTRODUCTION

Plants have been used to treat diseases such as diabetes, jaundices, cardiovascular diseases, heavy metal poisoning, congestion of abdominal and pelvic cavities and scarlet fever.^[1] It is estimated that out of 250,000 to 500,000 species of plants only 1 to 2% of the terrestrial plants have been reasonably well investigated. Although today the synthetic drugs are larger in their number than the natural ones, yet many synthetic drugs have their origin in the natural source and have been derived from plants and animals.^[2]

Liver, the key organ of metabolism and excretion, is constantly endowed with the task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents.^[3] Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequence. As such liver is highly affected primarily by toxic agents such as CCl₄,

paracetamol, D-galactosamine, alcohol, rifampicin and thioacetamide through different mechanisms.^[4]

The plant under study is *Andrographis paniculata*, commonly known as *Kalmegh* (king of bitter) and belongs to the family Acanthaceae.^[5] The plant has been reported to exhibit various mode of biological activities *in vivo*

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as well as *in vitro* viz., antibacterial,^[6] antiviral, anti-inflammatory,^[7] anti HIV (Human immunodeficiency virus), immunomodulation/ immunostimulatory and anticancer activities.^[8] The plant showed potential therapeutic action in curing liver disorders, common cough and colds in humans.^[9] It has astringent, diuretic, emmenagogue, gastric and liver tonic, carminative, antihelminthic, and antipyretic properties. Due to its “blood purifying” activity it is recommended for use in cases of leprosy, gonorrhoea, scabies, boils, skin eruptions, and chronic and seasonal fevers. The aim of the current study was to evaluate the hepatoprotective activity of the aqueous extract of *Andrographis paniculata* in wistar rats.

METHODS

Animals selected for the study

Animals: All the animals included in the study were procured from animal house of Mamata Medical College, Khammam. Laboratory bred wistar rats of either sex, weighing 100-200 gm were used for the study. The animals were maintained under standard laboratory conditions at 27-29°C. Experimental protocol has been approved by the Institutional Animal Ethics Committee.

Drugs used in the study

Carbon tetrachloride manufactured by Molychem and Tab. Liv 52 manufactured by The Himalaya Drug Company were used in the study.

Extraction procedure

The preparation of crude aqueous extract of leaves of *Andrographis paniculata* was done in the Department of Pharmacology, Mamata Medical College, Khammam. The extract was obtained from the powdered leaves by a process of Soxhlet extraction.

Experimental design

Wistar rats of both sexes weighing between 100-200 gm were used. Food was restricted 18 h prior to the experiment, but free access to water was allowed. The animals were divided into six groups with six rats in each group. All the animals were hydrated orally with 10 ml/kg of 0.9% normal saline for 7 days.

First group of six rats were taken as control, and they received 0.9% normal saline 10 ml/kg body weight orally. Second group of six rats was taken as CCl₄ control group

and treated with normal saline (10 ml/ kg, p.o.) daily for 20 days. CCl₄: olive oil (1:1, 2 ml/ kg, i.p.) on 2nd, 5th and 8th day, 30 min after administering of normal saline. The third group of six rats were treated with Liv 52 100 mg/ kg orally for 20 days and Carbon tetrachloride diluted with olive oil was given intra-peritoneally (1:1, 2 ml/ kg) on 2nd, 5th and 8th day, 30 mins after the administration of the standard drug.

The fourth and fifth groups were taken as test groups and they received crude aqueous extract of *Andrographis paniculata* obtained in liquid form along with normal saline orally once daily for 20 days in the dose of 250 and 500 mg/ kg respectively. Carbon tetrachloride diluted with olive oil was given intra-peritoneally (1:1, 2 ml/ kg) on 2nd, 5th and 8th day, 30 mins after the administration of the test drug.

Estimation of biochemical parameters

On the 21st day, all animals were anesthetized with mild ether and blood samples were collected by cardiac puncture method. The blood samples were collected separately from ventricle into sterilized dry centrifuge tube which was heparinised. The clear serum was separated at 2000 rpm for 15 min and biochemical investigations were carried out to assess liver function. Biochemical parameters namely serum SGOT, SGPT, ALT, bilirubine and cholesterol were assayed by standard methods.

Histopathology

The tissues of liver were removed from animals, washed with normal saline to remove blood, fixed in 10% formalin and embedded in paraffin wax. Sections of 5 µm thickness were made using rotary microtome and stained with haematoxylin-eosin and histological observations were made under light microscope.^[10]

Statistical analysis

Analysis of the data was done using one way ANOVA and Turkey test. P values of less than 0.05 were considered significant.

RESULTS

CCl₄ control group is treated with toxic doses of carbon tetra chloride had significantly elevated the values of the serum SGOT (310.4 ± 10.98), SGPT (256.4 ± 9.288), ALT (430 ± 3.225), cholesterol (185.8 ± 3.397), bilirubin direct (1.08 ± 0.07348) and bilirubin total (3.16 ± 0.2249) as compared to normal control. It indicates that acute

hepatocellular damage with CCl₄. Standard, Test-1 and Test-2 groups were treated with hepatotoxic dose of carbon tetrachloride followed by Liv-52 and different doses of *Andrographis paniculata* (250 & 500 mg/kg po). It showed that significantly lowered the values of the serum SGOT, SGPT, ALT, cholesterol, bilirubin direct and bilirubin total (P<0.05, P<0.01) as compared to CCl₄ control group as shown in Table 1 and 2.

Histological examination of liver sections of control group showed liver parenchyma with intact architecture. The periportal [Arrow, Figure 1a], perivenular and midzonal hepatocytes appear intact. The central vein [Arrow, Figure 1b] and sinusoids appear unremarkable. In CCl₄ intoxicated rats most of the perivenular and midzonal hepatocytes show steatosis and cytoplasmic vacuolations with ballooning degeneration [Short arrow, Figure 2a], the midzonal region shows inflammatory infiltration [Long Arrow, Figure 2a], the periportal region, central veins and sinusoids appear unremarkable were observed [Figure 2a & 2b]. The liver section of the rat treated with aqueous extract of different doses of *Andrographis paniculata* and Liv-52 followed by CCl₄ intoxication showed cellular regeneration that is a sign of protection as it was evident by the reduction of necrosis and sinusoids [Figure 3, 4 & 5].

DISCUSSION

Carbon tetrachloride-induced hepatic injury is commonly

Table 1: Effect of aqueous extract of *Andrographis paniculata* on serum SGOT, SGPT And ALT on CCl₄ induced hepatotoxicity

Groups	SGOT(U/L)	SGPT(U/L)	ALT(U/L)
Normal control (Normal saline 10ml/kg)	134 ± 5.099	60.2 ± 1.655	213.2 ± 9.478
CCl ₄ control (CCl ₄ 1.5 ml/kg)	310.4 ± 10.98	256.4 ± 9.288	430 ± 3.225
Standard (LIV-52 100mg/kg)	141.6 ± 5.784	98 ± 3.521	246.8 ± 4.684
Test-1 (<i>Andrographis paniculata</i> 250mg/kg)	192 ± 2.55*	185.6 ± 3.982*	297.2 ± 4.271*
Test-2 (<i>Andrographis paniculata</i> 500mg/kg)	166.4 ± 1.806†	139.6 ± 4.534†	265 ± 2.646†

All the values are represented as Mean ± SEM. * P < 0.05, † p < 0.001

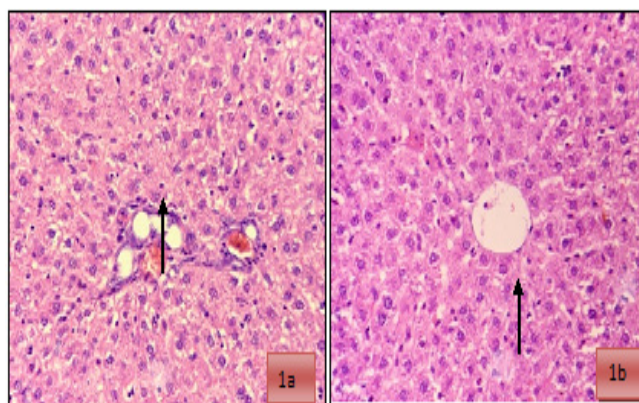


Figure 1: [Normal control] shows liver parenchyma with intact architecture. The periportal [Arrow, Figure 1a], perivenular and midzonal hepatocytes appear intact. The central vein [Arrow, Figure 1b] and sinusoids appear unremarkable.

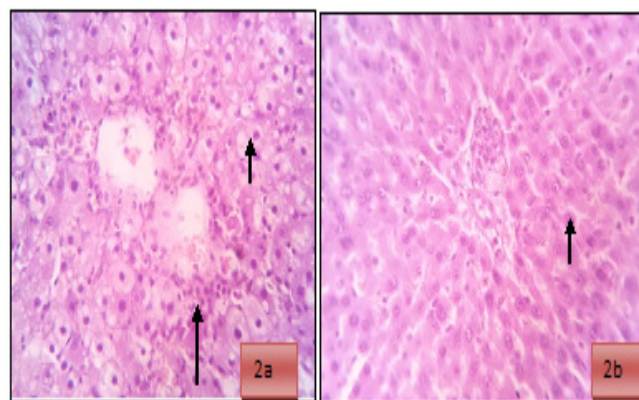


Figure 2: [CCl₄ control] shows liver parenchyma with intact architecture [Arrow, Figure 2b]. Most of the perivenular and midzonal hepatocytes show steatosis and cytoplasmic vacuolations with ballooning degeneration [Short arrow, Figure 2a]. The midzonal region shows inflammatory infiltration [Long Arrow, Figure 2a]. The periportal region, central veins and sinusoids appear unremarkable.

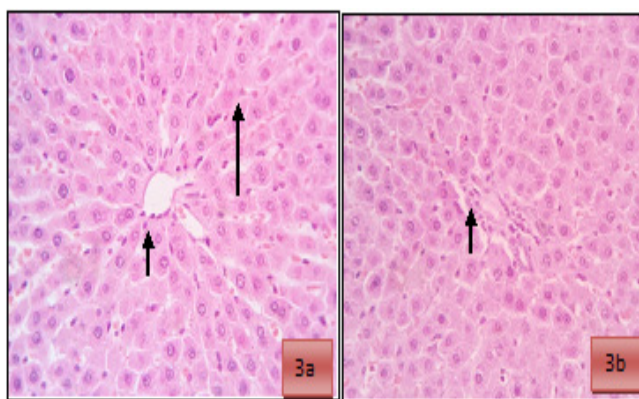


Figure 3: [standard] shows liver parenchyma with intact architecture [Figure 3a, Long Arrow]. The perivenular, periportal and midzonal hepatocytes appear intact. The periportal zone shows scant inflammatory infiltration [Short Arrow, Figure 3a]. The central veins [Arrow, Figure 3b] and sinusoids appear intact.

Table 2: Effect of aqueous extract of *Andrographis paniculata* on bilirubin and cholesterol on CCl₄ induced hepatotoxicity

Groups	Bilirubin direct (mg/dl)	Bilirubin total(mg/dl)	cholesterol (mg/dl)
Normal control (Normal saline 10ml/kg)	0.18 ± 0.03742	0.94 ± 0.04	120.4 ± 3.669
CCL4 control (CCL4 1.5 ml/kg)	1.08 ± 0.07348	3.16 ± 0.2249	185.8 ± 3.397
Standard (LIV-52 100mg/kg)	0.24 ± 0.02449	1.54 ± 0.153	127.4 ± 1.887
Test-1 (<i>Andrographis paniculata</i> 250mg/kg)	0.66 ± 0.06782*	2.8 ± 0.07071*	155.2 ± 3.967*
Test-2 (<i>Andrographis paniculata</i> 500mg/kg)	0.46 ± 0.04†	2.14 ± 0.07483†	137 ± 2.098†

All the values are represented as Mean ± SEM. * P < 0.05, † p < 0.001

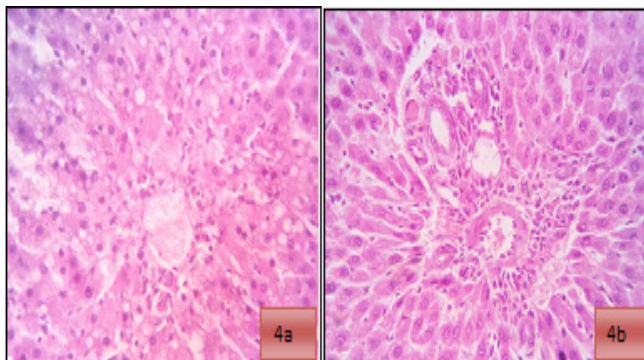


Figure 4: [Test-250mg/kg] shows liver parenchyma with intact architecture. Some of the perivenular and midzonal hepatocytes show mild steatosis with ballooning degeneration [Short arrow, Figure 4a]. The periportal region shows dense inflammatory infiltration [Arrow, Figure 4b]. The central veins appear congested [Long Arrow, Figure 4a] and sinusoids appear unremarkable.

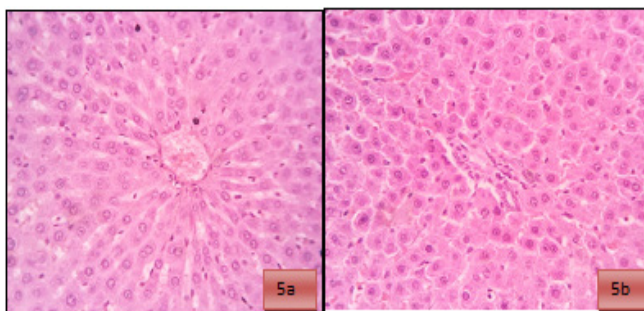


Figure 5: [Test-500mg/kg] shows liver parenchyma with intact architecture. The perivenular, periportal and midzonal hepatocytes appear unremarkable. The periportal region shows mild inflammatory infiltration [Long Arrow, Figure 5b]. The central veins [Arrow, Figure 5a] and sinusoids appear congested [Short Arrow, Figure 5b].

used as an experimental method for the study of hepatoprotective effect of drugs or medicinal plant extracts, by *In vivo* and *In vitro* techniques.^[11] Results indicated that the aqueous extracts of *Andrographis paniculata* provided significant protection against the toxic effect of CCl₄ on liver. Preventive action of liver damage induced by the CCl₄ has widely been used as indicator of the liver protective in general.^[12] CCl₄ produces an experimental damage that histologically resembles viral hepatitis.^[13] Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures.^[14] The toxic metabolite • CCl₃ radical is produced by microsomal oxidase system binds covalently to the macromolecule and causes per oxidative degradation of lipid membrane of the adipose tissue. However, when pre- treated with the test drug there was reduction in levels of SGOT and SGPT towards the respective normal values which is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl₄. This effect is in agreement with the commonly accepted view that serum level of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes.^[15] Alkaline phosphate (ALT) is the prototype of these enzymes that reflects the pathological alteration in biliary flow.^[16] CCl₄ induced elevation of this enzymatic activity in the serum is in line with high level of serum bilirubin content. The extract mediated suppression of the increased serum ALT activity with the concurrent depletion of raised bilirubin suggests the possibility of the test drug being able to stabilize biliary dysfunction in rat liver during hepatic injury. Histological studies reveal the changes, which take place during the damage and recovery. The extract mediated recovery supports the hepatoprotective activity of the same. The results of this investigation indicated that the aqueous extract of *Andrographis paniculata* possess significant hepatoprotective activity against CCl₄ induced liver damage in rats.

CONCLUSION

It is observed that there was significant decrease in SGOT, SGPT, ALT, bilirubine and total cholesterol at different doses of *Andrographis paniculata*. Thus, as this plant contains bitter glucosides: andrographolide, panaculoside, flavonoids, andrographonin, panicalin, neoandrographolide, apigenin 7-4-dimethyl ether, these phytochemicals may be responsible for hepatoprotective effect. *Andrographis paniculata* aqueous extract might efficiently increase the regenerative and reparative capacity of the liver.

CONFLICT OF INTEREST

Nil

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