



## HEPATOPROTECTIVE EFFECT OF *Solanum nigrum* LEAVE DIETHYL ETHER EXTRACT ON LIVER CCl<sub>4</sub> TOXICITY

Sheherzad Salman<sup>1</sup>, Rukhsana Anwar<sup>1\*</sup>, Rukhsana Sher<sup>1</sup>, Sairah Hafeez Kamran<sup>1</sup>, Asma Manzoor<sup>1</sup>

<sup>1</sup>University College of Pharmacy, University of the Punjab Lahore, Pakistan

### ABSTRACT

*Solanum nigrum* is a medicinal plant commonly known as black night shade and Makoi, it is found in two varieties. One has black colored fruits while the other has reddish brown colored fruit. Traditionally *Solanum nigrum* possesses a number of active compounds which are responsible for its diverse pharmacological properties. The current study aims to investigate the diethyl ether extract of *Solanum nigrum* leaves activity on the liver acute toxicity induced by carbon tetrachloride in rabbits. The rabbits were allocated randomly into two groups (n=6). The hepatic damage intensity and protection was observed by biochemically investigating the serum levels of Lactate dehydrogenase (LDH), bilirubin, Alanine transaminase (ALT), Aspartate transaminase (AST), alkaline phosphatase (ALP), total protein (TP) and tissue histopathology analysis. The results showed that the diethylether extract of *Solanum nigrum* leaves have significant ( $p < 0.05$ ) shielding effect on all hepatic enzymes and protein. Histopathological evaluation also confirmed that diethyl ether extract of leaves has potential to protect the liver against chemical (CCL<sub>4</sub>) induced injury.

**Keywords:** LDH, ALT, AST and Carbon tetrachloride

### INTRODUCTION

The WHO has defined traditional medicine as a field which encompasses traditional Chinese medicine, Indian ayurveda and Arabic unani medicine. They may be medication therapies such as those which require the use of herbal medicines or drugs, which may be animal parts or minerals, derived from plants, and they may be non-medication therapies that are those without the use of medicines like acupuncture or spiritual therapies (Pal and Shukla, 2003). significant anti-diabetic, immunostimulant activity (Edmonds and Chweya, 1997), antibacterial activity (Jain *et al.*, 2011), mild anti-fungal effects and anti-viral properties. These Studies have also revealed cardio-protective effect in dose dependent point of view of *S. nigrum* fruits. Other therapeutic uses include hypotensive, analgesic effects, cytotoxic, antidiarrhoeal, anti-inflammatory, anti-seizures and anticancer activities. *S. nigrum* contains a number of active compounds which are responsible for its multiple pharmacological properties (Yousaf *et al.*, 2006). Major

active constituents include glycoalkaloids, glycoproteins, and polysaccharides (Potawale *et al.*, 2008). In Africa, leaves were pounded and applied topically for the treatment of bacterial infections and warts. In Tunisia, Africa, the sap was used to treat acute streptococcal infections, snake bites, burns and dermal infections. In India, *S. nigrum* was used for the treatment of stomach ache, stomach ulcer, and liver tonic and also used to increase the fertility in women. Liver has the ability to perform different functions amongst which synthesis; metabolism and detoxification are major one. The liver has the ability to regenerate the damaged tissue (Tacke *et al.*, 2009). The common reason of liver failure is viral hepatitis A and B, metabolic disorders such as Reye's syndrome, ingestion of toxins such as amanita mushroom poisoning overdose of medication like acetaminophen and idiosyncratic drug reactions (Kaplowitz, 2000). An usual cause of liver inflammation is the hepatotoxicity caused by drug which may appear clinically as acute

\* Corresponding author: Rukhsana Anwar, University College of Pharmacy, University of the Punjab, Allama Iqbal Campus, Lahore-54000, Pakistan. Phone: +92-42-99211616. Email rukhsanaanwar2003@yahoo.com

hepatitis and/or cholestasis, however any form of acute or chronic liver disease can occur (Kaplowitz, 2004). Carbon tetra chloride (CCl<sub>4</sub>) has exert its harmful effects by the metabolism of liver's CYP450 enzymes which produces free radicals. The liver toxicity of CCl<sub>4</sub> can be managed by the partial pressure of oxygen in the tissues (Calvert and Brody, 1960). The following research study was conducted to determine the effect of diethylether extract of *Solanum nigrum* leaves on the hepatic distress induced by CCl<sub>4</sub>.

## MATERIALS AND METHODS

### Experimental Animals

The study was conducted on male healthy albino rabbits of the local strain *Albino* having a weight between 1 to 2 kg. The animals were purchased from local market in Lahore and were housed in the animal house of Punjab University College of Pharmacy, University of the Punjab, Lahore. The animals were kept in iron cages and were acclimatized for a period of one week before starting the study. During the entire study duration the animals were fed fresh green fodder twice a day and water *ad libitum*. The study protocol was approved by the animal ethical committee of University College of Pharmacy, University of the Punjab, Lahore and provided with voucher number AEC/UCP/1033/4313.

### Chemicals

Diethylether was purchased from sigma Aldrich. CCl<sub>4</sub>, Liquid paraffin, formalin and normal saline were purchased by MERCK. Diagnostic kits for ALT, AST, ALP, LDH, Bilirubin and total protein were supplied by Crescent Diagnostics, Saudi Arabia.

### Plant material

The leaves of *S.nigrum* were collected from outskirts of Lahore, Punjab. Approximately 12 kg of the fresh and infected free leaves of the plant was collected for the study. The whole plant was identified taxonomically and authenticated from the Herbarium of Department of Botany, Government College University, Lahore provided with voucher specimen (GC. Herb. Bot. 2422).

### Preparation of diethylether leaves extract of *Solanum nigrum*

The diethyl ether (DET) extract was prepared by soaking about 280 g in 800 ml of diethylether in flask at room temperature. The powder was soaked insolvent for 2 days and it was stirred regularly. The macerate was filtered using Whatman's filter paper no. 1 (Whatman Ltd., England). The entire process of maceration and filtrations was repeated 2 times with the solvent to get the maximum yield. To remove diethylether from the

distillate, the filtrate was fed into the vacuum rotary evaporator. The solvent was evaporated at temperature of 40°C by vacuum rotary evaporator. The extract was then dried in hot air oven at 37°C.

### Experimental design of *in vivo* acute study

The rabbits were categorized into two groups. Each group contained 6 rabbits (n=6), and all these rabbits were evaluated both physically and biochemically by taking their baseline data, before commencing the study. Group I Control: 0.5 mL/ Kg CCL<sub>4</sub> was administered. Group II: *S. nigrum* extract 2g/ Kg of body weight (b.wt) was administered orally followed by CCl<sub>4</sub> (0.5 ml/kg) orally.

The blood samples were collected prior to the administration of each dose of the extract or CCl<sub>4</sub>. After 4 hours, 8 hours and 24 hours of the administration of the single dose, blood samples were collected from the animal's ear vein and biochemical tests were performed.

### Biochemical Parameters Evaluation

Blood serum of each rabbit was prepared and ALT, AST, ALP, LDH, Bilirubin and Total protein was investigated by using Crescent Diagnostic Kits, Saudi Arabia.

### Histopathological Evaluation

After the 24 hour rabbits were sacrificed, the livers from each were removed. The livers were washed in cold saline, weighed and fixed in 11% formalin. The liver tissue was afterwards processed in different ethanolic percentages. Tissues were prepared for microtoming by adding xylene to harden them and were inserted in paraffin wax for 6 hours. Slices of liver tissue were fixed with gelatin and the slides stored at 52°C for 12 hours in the oven. Staining was then performed with hematoxylin and eosin (H & E) stains. Slides were covered with cover slip, sealed and finally were evaluated.

### Statistical analysis

The results are mean values  $\pm$  standard deviation (SD). The results were analyzed by applying one way ANOVA followed by Dunnett's test. The values were considered significant when  $p < 0.05$ .

## RESULTS

The CCL<sub>4</sub> at 0.5 ml/ kg caused the increase in AST concentration and it achieved significantly higher levels after 24 hours of its administration as shown in the Table 1. The ALT levels were affected by CCL<sub>4</sub> in such a way that this level kept on increasing gradually until at the 24<sup>th</sup> hour at which the ALT levels became significantly high. A significant amount of rise in the levels of ALP enzyme was observed during the 24 hour acute study

with CCl<sub>4</sub>. Significant amounts of increase were observed at 4 hour and 24 hours as shown in Table 1. The CCl<sub>4</sub> (0.5 mL/ kg) displayed increasing levels of LDH in the rabbits during the acute study period. The Table 1 shows that the CCl<sub>4</sub> (0.5 ml/ kg) after 24 hours achieved maximum high levels. CCl<sub>4</sub> showed no major effect on the total protein levels after its administration as shown in the Table 1. CCl<sub>4</sub> has significant effect on the bilirubin levels causing a rise at the 24<sup>th</sup> hour as compared to zero hour baseline reading. The bilirubin keeps increasing during each of the sampling interval and reaches a maximum at 24 hours. The Figure 2 shows the effect of CCL4 on bilirubin levels (mg/ dL).

Table 1: effects of CCl<sub>4</sub> on biochemical parameters of rabbit's serum (Group I) aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), bilirubin and lactate dehydrogenase (LDH) after CCl<sub>4</sub> induced acute hepatotoxicity.

Biochemical Parameters	Group I CCL4 induced rabbits			
	Time interval (hr)			
	0 (Control)	6	8	24
AST (IU/ L)	56.16 ± 5.3	80.07 ± 10.96	187.82± 116.22	545.24±81.74*
ALT (IU/ L)	65.66 ± 7.0	89.55 ± 5.3	150.84±84.85	716.82±82.58*
ALP (IU/ L)	73.61±15.93	277.73 ± 48.69*	293.93±64.76	556.04±55.89*
LDH (IU/ L)	96.31 ± 9.58	107.42 ± 4.42	132.21 ± 7.0	146.56±7.01*
Total protein (g/ dL)	7.96 ± 0.68	7.26 ± 0.21	8.03 ± 0.41	7.56 ± 0.71
Bilirubin (mg/ dL)	0.4 ± 5.55	0.63 ± 0.09	0.5 ± 0.14	1.03 ± 0.28*

Analysis was done by using of One way ANOVA followed by Dunnett's test.

The diethylether extract of *S. nigrum* leaves prevented a significant elevation in the levels of AST when administered one hour prior to the CCl<sub>4</sub>. The diethylether extract of *S. nigrum* leaves ameliorate the serum level of ALT enzyme which suggests that the extract has hepatoprotective in rabbits. The diethylether extract of *S. nigrum* leaves showed considerable serum ALP lowering effect at the 24<sup>th</sup> hour. Diethyl ether extract of *S. nigrum* leaves displayed significant LDH lowering effect at the 4<sup>th</sup> and 8<sup>th</sup> hour of CCl<sub>4</sub> administration.

Analysis was conducted by using one way ANOVA followed by Dunnett's test. No significant effect of the diethyl ether extract of *S. nigrum* leaves was observed on the serum total protein levels of the rabbits as shown Table 2. The bilirubin level did not elevate significantly

in the presence of the diethylether *S. nigrum* leaves extract.

The hepatic parenchyma, hepatic cells and central vein in control group was shown to be normal, as shown in Figure 1. The Figure 2 shows the liver damaged by the administration of CCl<sub>4</sub>.

Table 2: effects of *Solanum nigrum* leaves diethylether extract on rabbits serum (Group II) biochemical parameters aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), bilirubin and lactate dehydrogenase (LDH) after CCl<sub>4</sub> induced acute hepatotoxicity.

Biochemical parameter	Group II diethyl ether extract (2g/Kg b.wt) + CCL4			
	Time interval (hr)			
	0 (Control)	6	8	24
AST (IU/ L)	60.56±11.97	86.96±8.45	69.1 ± 7.78	51.54±5.22
ALT (IU/ L)	55.63±17.76	86.57±14.33*	71.2±17.37	60.04±17.91
ALP (IU/ L)	70.91±10.18	74.17±5.25	70.38 ± 8.61	67.86±11.71
LDH (IU/ L)	171.79±16.86	131.95±22.64*	132.83 ± 23.01*	159.69±13.35
Total protein (g/ dL)	7.9±0.75	7.16±0.39	7.26±0.65	6.46±0.53
Bilirubin (mg/ dL)	0.5 ± 0	0.33±0.04	0.4 ± 0.21	0.26±0.16

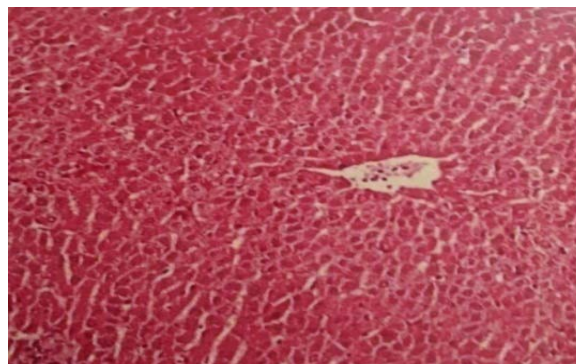


Figure 1: The hepatic parenchyma, hepatic cells and central vein in control group.

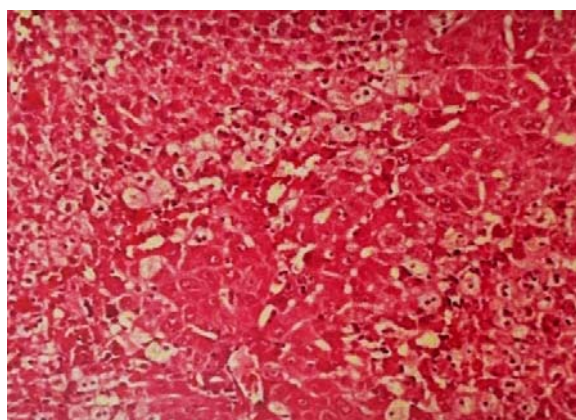


Figure 2: The liver damaged by the administration of CCl<sub>4</sub>.

The hepatic parenchyma is damaged, necrosis and infiltration is shown in Figure 3: The hepatic epithelial cells show mirror necrosis in treated group. Normal morphology of rabbit's liver shows normal hepatic cells and normal central vein as shown in Figure 1. The hepatic parenchyma is damaged by showing necrosis and infiltration. Fatty changes are also observed. The livers had undergone treatment with the diethyl ether extract and CCl<sub>4</sub> as shown in Figure 3.

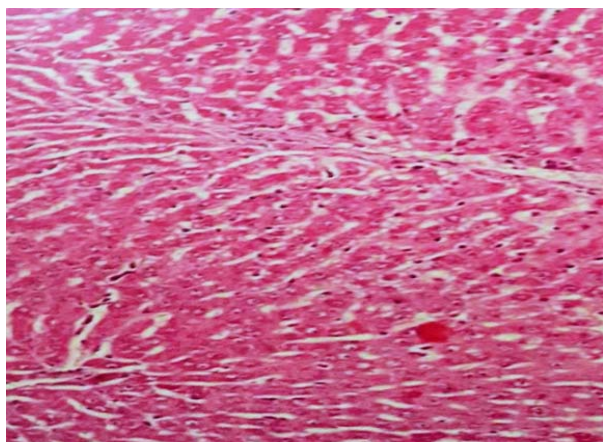


Figure 3: The hepatic parenchyma showing restored parenchyma in diethyl ether extract and CCl<sub>4</sub> treated group

## DISCUSSION

The water and methanolic extracts of *S. nigrum* have hepatoprotective effect against CCl<sub>4</sub> intoxicated rats (Elhag and ElBadwi 2015). No study is available to investigate the effects of leaves extracted in diethyl ether (non-polar solvent). This study was carried out to evaluate the hepatoprotective activity of *S. nigrum* leaves extract.

Carbon tetrachloride (CCl<sub>4</sub>) has been used to induce damage in liver. It causes liver toxicity by an elevation in the serum enzyme levels of ALT, AST and ALP (Raju *et al.*, 2003). Various liver function tests were performed to analyze the extent to which hepatoprotection was achieved.

The serum bilirubin level rose insignificantly in diethyl ether extract treated group as compared to chemical injury produced by CCl<sub>4</sub>. The liver markers for injury are mainly ALT, AST and bilirubin. The diethyl ether extract of *S. nigrum* kept the level of serum ALT steady in the treated group. The presence of active constituents in the diethyl ether extract of *S. nigrum* may responsible for masking the effect of CCl<sub>4</sub> on serum ALT activity.

The levels of serum AST in rabbits treated with diethyl ether extract of *S. nigrum* prior to administration of CCl<sub>4</sub> showed minor elevations as compared to CCl<sub>4</sub> treated group (Table 1). The diethyl ether extract of *S. nigrum* displayed hepatoprotective activity by considerably lowering the AST levels.

Serum ALP level increases with the administration of CCl<sub>4</sub>. The membrane integrity of cell membrane is lost and ALP enzyme enter into the circulation. CCl<sub>4</sub> causes increased level of serum ALP as observed in the control group. Serum ALP level reduced significantly at the 4<sup>th</sup>, 8<sup>th</sup> and 24<sup>th</sup> hour in the treated group as compared to control group. The total protein levels could not changed significantly with any extracts of *S. nigrum*. The livers of normal rabbits showed no histological change. In the CCl<sub>4</sub> treated group, hepatic lobules become damage. Hepatic degeneration and enlargement of central vein was visible. There was focal infiltration of cells near to central vein. Mild fatty change in hepatocytes was also observed. The diethyl ether extracts treated group showed protection against CCl<sub>4</sub> damage.

## CONCLUSION

The diethyl ether extract of *S. nigrum* leaves showed hepatoprotective activity effect mediated by lowering the serum ALT, ALP, AST and bilirubin serum level as compared to treated with CCl<sub>4</sub>. These effects can be attributed that *Solanum nigrum* leaves have the capability to scavenging free radicals. Further studies need to be carried out to elucidate the mechanism of action as a hepatoprotective agent.

## REFERENCES

- Calvert, D. N. & Brody, T. M. 1960. Role of the sympathetic nervous system in CCl<sub>4</sub> hepatotoxicity. *American Journal of Physiology--Legacy Content*, 198, 669-676.
- Edmonds, J. M. & Chweya, J. A. 1997. *Black nightshades: Solanum nigrum L. and related species*, Bioversity International.
- Jain, R., Sharma, A., Gupta, S., Sarethy, I. P. & Gabrani, R. 2011. *Solanum nigrum*: current perspectives on therapeutic properties. *Altern Med Rev*, 16, 78-85.
- Kaplowitz, N. 2000. Mechanisms of liver cell injury. *Journal of hepatology*, 32, 39-47.
- Kaplowitz, N. 2004. Drug-induced liver injury. *Clinical infectious diseases*, 38, S44-S48.
- Pal, S. K. & Shukla, Y. 2003. Herbal medicine: current status and the future. *Asian pacific journal of cancer prevention*, 4, 281-288.
- Potawale, S., Sinha, S., Shroff, K., Dhalawat, H., Boraste, S., Gandhi, S. & Tondare, A. 2008.

- Solanum nigrum Linn: A phytopharmacological review. *Pharmacologyonline*, 3, 140-163.
- Raju, K., Anbuganapathi, G., Gokulakrishnan, V., Raj Kapoor, B., Jayakar, B. & Manian, S. 2003. Effect of dried fruits of Solanum nigrum LINN against CCl<sub>4</sub>-induced hepatic damage in rats. *Biological and Pharmaceutical Bulletin*, 26, 1618-1619.
- Tacke, F., Luedde, T. & Trautwein, C. 2009. Inflammatory pathways in liver homeostasis and liver injury. *Clinical reviews in allergy & immunology*, 36, 4-12.
- Yousaf, Z., Masood, S., Shinwari, Z. K., Khan, M. A. & Rabani, A. 2006. Evaluation of taxonomic status of medicinal species of the genus Solanum and Capsicum based on Poly Acrylamide Gel Electrophoresis. *Pakistan Journal of Botany*, 38, 99.