Ranu Yadav *et al*, International Journal of Pharmaceutical Sciences & Medicine (IJPSM),
Vol.8 Issue. 3, March- 2023, pg. 123-131

ISSN: 2519-9889
Impact Factor: 5.9

Hepatoprotective Effect of Methanolic Leaf Extract of *Prosopis Cineraria* on Paracetamol Induced Hepatotoxicity in Swiss Albino Mice

Ranu Yadav[1]; Dr. P.K. Dubey[2]; Anant K. Patel[3]
[1]Student, Swami Vivekanand College of Pharmacy (M. Pharm Pharmacology)
[2]Principal, Swami Vivekanand College of Pharmacy
[3]Associate Professor, Swami Vivekanand College of Pharmacy

DOI: 10.47760/ijpsm.2023.v08i03.009

Abstract

Aim: To evaluate the protective effect of methanolic leaf extract of *Prosopis Cineraria* on paracetamol-induced hepatotoxicity in mice. **Materials and Methods:** 30 Swiss albino mice of eithersex mice were used in this study and divided into 5 groups (for each 6). Group 1 Control group fed basal diet and maintained as positive control group. Group 2 received Paracetamol in a dose of (3 g/kg b. wt.). Group 3 received Paracetamolalong with Standard Drug (Silymarin) in a dose of (100 mg/kg). Groups 4 and 5 were treated with Paracetamol along with plant extracts of *Prosopis Cineraria* at a dose level of 250 g/kg and 500 mg/kg. **Results:**The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglyceride (TG), bilirubin, and total protein were measured. The values of urea, sodium, potassium and chloride were significantly increased in rats exposed to paracetamol. Moreover, administration of paracetamol resulted in damage to liver structures. Administration of methanolic extract of *Prosopis Cineraria* before Paracetamol exposure prevented severe alterations of biochemical parameters and disruptions of liver structures. **Conclusions:** This study obviously demonstrated that pre-treatment with methanolic extract of *Prosopis Cineraria*significantly attenuated the physiological and histopathological alterations induced by paracetamol.

Introduction

The liver is a essential organ in energy uptake and the biotransformation of xenobiotics. Therefore, recurrent exposure to toxic xenobiotics is likely to aggravate a liver injury, resulting in cirrhosis, liver cancer, and acute liver failure [1]. Paracetamol (Acetaminophen or N-acetyl-para-aminophenol (APAP)) is one of the maximumsextensively used analgesics and antipyretic drugs universal. Although considered safe at therapeutic doses (up to 4000 mg/day), Paracetamol, at higher doses, can induce centrilobular necrosis which commonly leads to a fatal outcome [2]. Paracetamol intoxication would be responsible for about one-half of all cases of acute liver failure in the United States and the United Kingdom [3]. Paracetamol hepatotoxicity is begun by the production of N-acetyl-p-quinone-imine (NAPQI), a volatile metabolite generated by cytochrome P450 enzymes that metabolize the drug when existing at high doses. This compound then drains glutathione stores

ISSN: 2519-9889
Impact Factor: 5.9

and binds to several cellular proteins exclusively mitochondrial proteins, thereby leading to mitochondrial oxidant stress which origins cell death [4]. Moreover, it was revealed that the immune system plays a role in the development of Paracetamol-induced hepatotoxicity. Indeed, activated Kupffer cells produce some proinflammatory and chemotactic cytokines which promote infiltration of neutrophils and macrophages in the liver tissue, causing therefore an exacerbation of liver damage [5]. To block this toxicity, coadministration of N-acetylcysteine (NAC), a cysteinederived antidote, is often useful, but some side effects, such as hypotension, limit its worth [6]. Consequently, the search for novel liver-protective agents is necessary to reinforce the existing therapeutic arsenal.

Material and Method

Collection and authentication of plant

The plant was collected from Pharmacognosy Garden of College of Swami Vivekanand College of Pharmacy, Khandwa Road, Indore, Madhya Pradesh, India. The plant was authenticated by Dr. S.N. Dwivedi, Department of Botany Janata PG College, A.P.S. University, Rewa, Madhya Pradesh, India.

Preparation of plant extract

The fresh leaves of *prosopis cineraria* were cleaned, shade dried and the powderedand then dried in an oven below 60 ºC. The dried leaves of *prosopis cineraria* were then pulverized into coarse powder in a grinding machine. The dried coarse powder of the *prosopis cineraria* leaves (1000 g) was extracted by using the Soxhlet apparatus to perform methanolic extraction. The methanolic extract of *prosopis cineraria* is then concentrate on a water bath and dried under reduced pressure to achieve a dark brown mass [7].

Phytochemical Analysis

The methanolic extractof *prosopis cineraria* were subjected to the phytochemical analysis using conventional protocol like alkaloids, carbohydrates, glycosides, flavonoids, saponins, tannins, protein, amino acids etc.

Experimental animals

A study was carried out by usingSwiss albino mice of either sex, weighing 20 g -25 g. They were obtained from the animal house. The animals were grouped and housed in polyacrylic cages, six animals per cage. Animals were housed at a temperature of 24 ± 2 ºC and relative humidity of 30-70%. A 12/12 h light and dark cycle was followed. The animals were nourished with standard pellet diet and fresh water ad libitum. All the animals were adapted to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the Institutional Animal Ethics Committee.

ISSN: 2519-9889
Impact Factor: 5.9

Evaluation of hepatoprotective activity

Paracetamol induced liver damage in mice was used as model. All the animals were divided into the five groups; each group consisted of 6 animals and they received the treatment as follows:

Group I: Control (1 ml/kg Saline orally)

Group II: Paracetamol (3 g/kg orally) for 7 days

Group III: Silymarin (100mg/kg) + Paracetamol for 7 days

Group IV: Methanolic extract of*Prosopis Cineraria* (250 mg/kg orally) + Paracetamol for 7 days

Group V: Methanolic extract of *Prosopis Cineraria*(500 mg/kg orally) + Paracetamol for 7 days

Biochemical estimation

At the end of experimental period, mice were anaesthetized with ether. Blood samples were collected from retro orbital venous plexus in nonheparinized tubes, centrifuged at 3000 rpm for 20 minutes, and blood sera were collected and stored at 4 ºC prior immediate determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), direct bilirubin (DB), total bilirubin (TB) total protein, urea, sodium, potassium and chloride.

Histopathological Examination

For light microscopic examination, liver tissues from each group were fixed with 10% buffered formalin, embedded with paraffin. After routine dealing out, paraffin sections of each tissue were expurgated into 4 µm thickness and discoloured with haematoxylin and eosin.

Statistical Analysis

All the data expressed as mean ± S.E.M and analysed statistically using ANOVA followed by Dunnett test and compared with respective control group. A value of $p < 0.05$ was considered significant.

Results

Phytochemical investigation

Methanolic extract of *Prosopis Cineraria*showed the presence of alkaloids, saponin glycosides, amino acids, tannins, terpenoids, steroids and flavonoids in both extracts.

ISSN: 2519-9889
Impact Factor: 5.9

Acute toxicity:

Methanolic extractsof *prosopis cineraria* did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose.

Hepatoprotective activity

The hepatoprotective activity of methanolic extracts of *Prosopis Cineraria*on Paracetamol treated mice are shown in Table 1. Increase in the serum hepatic enzyme levels, such as SGOT, SGPT, SALP, LDH, TB and GGT by the administration of paracetamol (3 g/kg, orally) was compared to normal groups. While the other parameters such as albumin and total protein showed the marked reduction in the levels of liver enzymes as compared to the normal groups indicating liver damage. Body weight and liver weight also showed the significant results that indicate chronic liver damage Table 2.

Histopathological results

Microscopic examination of the liver in normal group with features of polygonal nucleus with nucleolus, abundant cytoplasm and bilobed nucleus and showed no visible changes, disarrangement in hepatic cells (Figure 1). These normal structures were absent in toxic control group in which mice treated with paracetamol showed many severe histopathological alterations. Administration of paracetamol for seven days resulted in the impairment of liver structure along with messiness of hepatic strands. Several cells also showed histological features of necrosis. Moreover, an enlargement of the sinusoids and vacuole formations in hepatocytes, leucocytic infiltrations, dilation, and congestion of blood vessels with haemorrhage were noted in liver of rats exposed to paracetamol(Figure 2). The histopathological profile of the mice treated with methanolic extract of *Prosopis Cineraria* brought back the cellular arrangement around the central vein and reduced necrosis (Figs 1C and 1D). Also, it facilitated to bring the blood vessels to normal form. (Figure 4). While the standard group treated with Silymarin along with paracetamol showed less disarrangement and degeneration of hepatocytes, indicating marked regeneration activity (Figure 3)

ISSN: 2519-9889
Impact Factor: 5.9

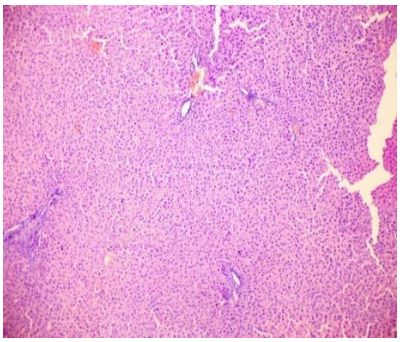

Figure 1

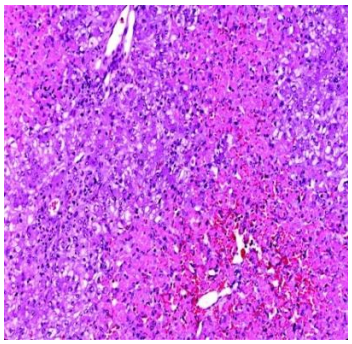

Figure 2

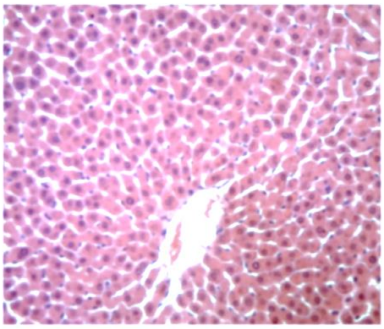

Figure 3

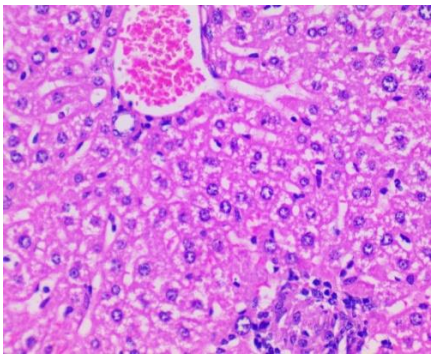

Figure 4

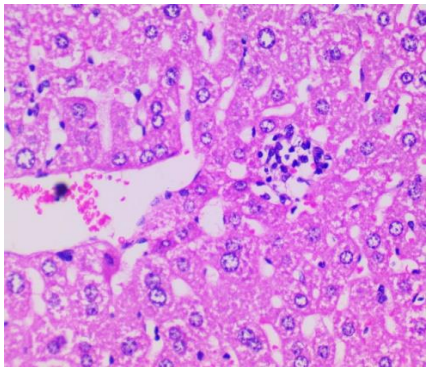

Figure 5

Figure 1-5: Histological monograph of extract and standard 1. Normal; 2. Paracetamol (3g/kg); 3. Silymarin (mg/kg) 4. Paracetamol + Methanolic extract (250 mg/kg); 5. Paracetamol + Methanolic extract (500 mg/kg).

Table No. 1: Level of ALP, AST, ALT, Total protein, Total bilirubin & Albumin in normal, induced, standard & treated mice.

S. No.	Groups	AST (mg/dl)	ALT (mg/dl)	ALP (mg/dl)	Albumin (mg/dl)	Total Protein (mg/dl)	T.B. (mg/dl)
1.	Control Group	84.12 ± 2.02	92 ± 2.32	143.35 ± 2.37	1.95 ± 0.37	4.05 ± 0.37	0.95 ± 0.89
2.	Untreated Group (Paracetamol)	209.45 ± 7.12	225.07 ± 5.06	320.94 ± 1.98	1.85 ± 0.15	3.10 ± 0.15	3.23 ± 0.49

ISSN: 2519-9889
Impact Factor: 5.9

3.	Standard Group (Silymarin)	75.73 ± 6.84	84.45 ± 4.56	139.94 ± 3.56	2.05 ± 0.39	4.01± 0.21	1.11 ± 0.37
4.	Extract (250 mg/kg) + Paracetamol	109.70 ± 5.62	115.30 ± 4.07	185.26 ± 3.22	2.10 ± 0.54	4.95 ± 0.46	1.09 ± 016
5.	Extract (500 mg/kg) + Paracetamol	80.25 ± 1.37	100.57 ± 5.78	154.65 ± 3.54	2.15 ± 0.43	5.01 ± 0.85	1.15 ± 0.93

Table No. 2: Effect of ethanolic extract of *Prosopis Cineraria* on change of body and liver weight in Paracetamol treated mice.

S.no.	Groups	Initial Body Weight	Change of Body Weight	Initial Liver Weight	Liver Weight
1.	Control Group	24 ± 0.53	24 ± 0.53	1.92 ± 0.86	1.92 ± 0.86
2.	Untreated Group (Paracetamol)	25 ± 1.15	25 ± 1.15	3.86 ± 0.37	3.86 ± 0.37
3.	Standard Group (Silymarin)	24± 0.23	24± 0.23	2.50 ± 0.21	2.50 ± 0.21
4.	Extract (250 mg/kg) + Paracetamol	24 ± 0.57	24 ± 0.57	3.49 ± 0.27	3.49 ± 0.27
5.	Extract (500 mg/kg) + Paracetamol	23 ± 0.98	23 ± 0.98	3.01 ± 0.06	3.01 ± 0.06

DISCUSSION

Paracetamol is a common analgesic and antipyretic drug. Several studies have indicated the induction of hepatocellular damage or necrosis acetaminophen higher doses in experimental animals and humans [8]. For screening of hepatoprotective agents, paracetamol-induced hepatotoxicity has been used as a well-founded method. Paracetamol is metabolized primarily in the liver and eliminated by conjugation with sulphate and glucuronide, and then excreted by the kidney. Additionally, paracetamol hepatotoxicity has been ascribed to the development of toxic metabolites, when a part of paracetamol is activated by hepatic cytochrome P-450 to a

ISSN: 2519-9889
Impact Factor: 5.9

extremely reactive metabolite N-acetyl-p-benzoquinone mine (NAPQI) [9]. Toxic metabolites (N-acetyl-p-benzoquinone mine) can alkylate and oxidize intracellular GSH, which results in liver GSH depletion subsequently leads to increased lipid peroxidation by abstracting hydrogen from due to higher doses of paracetamol. [10] Reactive metabolites can exert initial cell stress through a wide range of mechanisms including depletion of glutathione (GSH) or binding to enzymes, lipids, nucleic acids and other cell structures [11]. AST predominantly found in mitochondria of hepatocytes. ALT is more definite to the liver, and thus is a better parameter for detecting liver injury. Serum ALP and bilirubin are also associated with liver cell damage. The ALT, AST and ALP activity and serum bilirubin level are largely used as most common biochemical markers to evaluate liver injury [12, 13,]. Administration of paracetamol caused a significant ascent of enzymes level such as AST, ALT, ALP and bilirubin level has been imputed to the damage structural integrity of liver, because they are cytoplasmic in location and released into circulation after cellular damages indicating development of hepatotoxicity [14]. The co administrations of all examined plant extract have prevented the increased serum marker enzymes AST, ALT, ALP level and bilirubin level. This is in arrangement with the commonly recognized view that serum levels of AST, ALT and ALP return to normal with the curative of hepatic parenchyma and the regeneration of hepatocytes [15]. Decrease of serum albumin in paracetamol treated group may be due to development of protein adduct. Toxic Metabolites NAPQI leads to covalent modification of cellular target protein, cell death and organ damage [16]. Studied plants, *Prosopis Cineraria* exhibit the excellent hepatoprotective properties as indicated by maximum prevention of increased serum biochemical parameters of paracetamol induced toxicity. Catalase converts harmful hydrogen peroxide into water and oxygen and protects the tissues from highly reactive hydroxyl radicals [17]. The reduction in the activity of this enzyme may result in a number of deleterious effects due to accumulation of highly toxic metabolites and hydrogen peroxide on paracetamol administration, which can induce oxidative stress in the cells [11]. Co-administration of *Prosopis Cineraria* increases the activities of catalase in animals to prevent the accumulation of excessive free radicals and protects the liver from paracetamol intoxication. The increase in TBARS level in the liver induced by paracetamol suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms. GSH detaches free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. The GSH diminution in hepatic mitochondria is considered the most important mechanism in the paracetamol induced hepatotoxicity. Reduced GSH level was depleted in paracetamol treated group may be due to conjugation of GSH with NAPQI to form mercapturic acid [10]. In present study, studied plants, *Prosopis Cineraria* has greatest ability to reduce oxidative stress by increasing glutathione level and preventing lipid peroxidation in compared to other studied plants. Studied plant extract contains antioxidants and hepatoprotective activity through regulatory action on cellular permeability, stability and suppressing oxidative stress. A number of scientific reports indicated that certain flavonoids, triterpenoids and steroids have protective effects on the liver due to its antioxidant properties [17]. Phytochemically, *Prosopis*

ISSN: 2519-9889
Impact Factor: 5.9

Cineraria contains triterpenes, flavonoids, sesquiterpene and sesquiterpene evoninoate alkaloids might play a role in hepatoprotective activity. [18] From our results, it can be concluded that decreased levels of GSH, catalase activity, and increased serum marker enzymes and lipid peroxidation level in paracetamol treated rats was due to hepatocellular damage. Extract of *Prosopis Cineraria* afforded protection from such paracetamol induced liver damage.

CONCLUSION

In this study, hepatoprotective activity of the aqueous extract of *Prosopis Cineraria* was studied. The aqueous extract of *Prosopis Cineraria* at the dose of 500 mg/kg showed very prominent and similar to silymarin hepatoprotective activity as demonstrated by significant ($P<0.05$) decrease in transaminase enzyme levels and preserved the structural integrity of the hepatocellular membrane. Identification of natural compounds of plants will help to develop new therapeutically agents. The results obtained from present study shows that this plant is a good natural source for hepatoprotective activity. As this plant is easily available and the aqueous extract is showing better activity, this suggests that this plant is a cost-effective natural treatment available in the market. Further clinical trials should be done in order to develop a prominent formulation that will be useful for the public. As the cost of the treatment is rising, developing cost effective remedies will definitely give a better option and opportunities to treat chronic disease

REFERNCES

[1]. J. G. Kenna, "Mechanism, pathology, and clinical presentation of hepatotoxicity of anaesthetic agents," in Drug-Induced Liver Disease, pp. 403–422, Academic Press, 2013.
[2]. J. A. Hinson, D. W. Roberts, and L. P. James, "Mechanisms of acetaminophen-induced liver necrosis," Handbook of Experimental Pharmacology, vol. 196, pp. 369–405, 2010.
[3]. A. M. Larson, J. Polson, R. J. Fontana et al., "Acetaminophen-induced acute liver failure: results of a United States multicentre, prospective study," Hepatology, vol. 42, no. 6, pp. 1364–1372, 2005.
[4]. N. Pholmoo and C. Bunchorntavakul, "Characteristics and outcomes of acetaminophen overdose and hepatotoxicity in Thailand," Journal of Clinical and Translational Hepatology, vol. 7, pp. 1–8, 2019.
[5]. H.-C. Lee, H.-P. Yu, C.-C. Liao, A.-H. Chou, and F.-C. Liu, "Escin protects against acetaminophen-induced liver injury in mice via attenuating inflammatory response and inhibiting ERK signalling pathway," American Journal of Translational Research, vol. 11, no. 8, pp. 5170–5182, 2019.
[6]. S. E. Owumi, J. P. Andrus, L. A. Herzenberg, and L. A. Herzenberg, "Co-administration of N-acetylcysteine and acetaminophen efficiently blocks acetaminophen toxicity," Drug Development Research, vol. 76, no. 5, pp. 251–258, 2015.
[7]. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 26th edition. India, NiraliPrakashan, 2004, pp 101-10.
[8]. Vermeulen, N.P.E., Bessems, J.G.M. and Vandestraat, R.,"Molecular aspects of paracetamol induced hepatotoxicity and its mechanism -based prevention" Drug Metabolism Reviews., 24: 367-407 (1992)
[9]. Savides, M.C., Oehme, F., Nash S.L., and Leipold H.W., "The toxicity and biotransformation of single doses of acetaminophen in dog and cats" Toxicology and Applied Pharmacology., 3: 95-111 (1983).

ISSN: 2519-9889
Impact Factor: 5.9

[10]. Mitchell, J.R., Jollow, D.J, Potter, W.Z., Gillette, J.R. and Brodie, B.B. "Acetaminophen induced hepatic necrosis. I. Role of covalent binding in vivo" Journal of Pharmacology and Experimental Therapeutics 187: 211- 217(1973).

[11]. Pauli-Magnus, C., Stieger, B., Meier, Y. Kullak-Ublick, G.A. and Meier, P.J. "Enterohepatic transport of bile salts and genetics of cholestasis" Journal of Hepatolology.,43(2): 342-357 (2005).

[12]. Kozer, E., Evans, S., Barr, J., Greenberg, R., Soriano I, Bulkowstein, M, Petrov, I., Chen-Levi, Z., Barzilay, B. and Berkovitch, M., "Glitathione- dependent enzymes and antioxidant status in erythrocytes from children treated with high-dose paracetamol" British Journal of Clinical Pharmacology, 55(3): 234- 40 (2003).

[13]. Girish, C., Koner, B.C., Jayanthi, S., Rao, K.R., Rajesh, B. and Pradhan, S.C. "Hepatoprotective activity of six polyherbal formulations in paracetamol induced liver toxicity in mice" Indian Journal of Medical Research, 129(5): 569- 578 (2009).

[14]. Sallie, R., Tredger, J.M. and William, R. "Hepatoprotective, DNA damage prevention of Spirulina Platensis on CCL4 induced hepatotoxicity in mice" Biopharmaceutics Drug Disposal., 12: 251-259 (1991)

[15]. Thabrew, M. and Joice, P., "A comparative study of the efficacy of Pavette indica and Osbeckiaoctanda in the treatment of liver dysfunction" Planta Medical., 53: 239–241 (1987).

[16]. Zaher, H., Buters, J.T, Ward, J.M., Bruno, M.K., Lucas, A.M., Stern, S, T, Cohen, S.D., and Gonzalez, F.J., "Protection against acetaminophen toxicity in CYP1A2 and CYP2E1double null mice" Toxicology Applied Pharmacology, 152(1): 193-9 (1998).

[17]. Parmar, S.R., Dave, G.S., Patel, H.V. and Kalia K., "Hepatoprotective value of some plants extract against CCL4 toxicity in male rats" Journal of Cell and Tissue Research., 9(1): 1737- 1743 (2009).

[18]. Gupta, A.K. and Misra, N. "Hepatoprotective activity of aqueous ethanolic extract of chamomile capitula in paracetamol intoxicated albino rats" American Journal of Pharmacology and Toxicology, 1: 17-20 (2006).

