IJPSR (2020), Volume 11, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 July 2019; received in revised form, 03 November 2019; accepted, 08 February 2020; published 01 May 2020

ETHANOLIC LEAF EXTRACT OF *PREMNA TOMENTOSA* AMELIORATES ALCOHOL INDUCED HEPATOTOXICITY IN ALBINO RATS

T. Gomathipriyadharsini [*1], M. Kavimani [2], S. Sathiyanarayanamurthy [1] and K. Prabhu [2]

Bharath University [1], Department of Anatomy, Vivekanandha Dental College for Women, Thiruchengode, Namakkal - 637205, Tamil Nadu, India.
Sree Balaji Medical College and Hospital [2], Chennai - 600044, Tamil Nadu, India.

Keywords:

Premna tomentosa, Hepatotoxicity, Liver enzymes, Liver weight, Ethanol

Correspondence to Author:
Dr. T. Gomathipriyadharsini

Research Scholar,
Bharath University,
Department of Anatomy,
Vivekananda Dental College for Women, Thiruchengode, Namakkal - 637205, Tamil Nadu, India.

E-mail: dr.gomathi1985@gmail.com

ABSTRACT: Alcoholic liver disease (ALD) comprises a wide variety of damage, starting from steatosis to liver cancer. ALD results from a multifactorial interaction between behavioral, ecological and hereditary factors. This study aimed to evaluate the hepatoprotective effect of ethanolic extract of *Premna tomentosa* (EEPT) on ethanolic toxicity liver. The characterization of EEPT extract was performed using standard biochemical analysis. Thirty-six male albino Wistar rats were divided into 6 groups; Hepatotoxicity was induced using 40% ethanol (1ml/100gm b.w) for 10 days. Group I received distilled water. Group II, III, IV, V were administered with 40% ethanol (1 ml/100gm); Group III and IV was given EEPT (500 and 750 mg/kg, b.w), Group V receives Ayurvedic drug (Liv 52 1ml/100gm) and Group VI was given EEPT (500 mg/kg) for forty-five days. Total body weight, wet weight of the liver, serum total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase was compared and assessed statistically. EEPT of 500 and 750 mg/kg shows a significant ($p<0.01$) reduction of liver enzymes, it shows the protection against ethanol-induced hepatotoxicity.

INTRODUCTION: Ethanol is the psychoactive compound present in alcoholic drinks [1]. Alcohol, the most commonly consumed xenobiotic [2], leads to increased liver oxidative stress through the generation of highly reactive oxygen species (ROS) and adducts [3]. Alcoholic liver disease (ALD) is a major health problem worldwide [4]. ALD typically progresses through the stage of alcoholic stetosis, alcoholic hepatitis and alcoholic cirrhosis [5]. According to the World Health Organization (WHO), 80% of the emerging world's population relies on the use of herbal medicines [6, 7].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(5).2411-14

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(5).2411-14

Various medicinal plants such as *Eclipta prostrate*, *Emblica myrobalan*, *Phyllanthus niruri*, *Erthrina indica etc*., have shown good hepatoprotective property [8]. One such plant *Premna tomentosa* L. (Verbenaceae), commonly called Pidanganari in Tamil is used extensively in the Indian traditional system of medicine against liver, spleen and stomach disorders [9]. It is a moderately sized deciduous tree widespread in India along the coastal regions and hills. Extracts from *P. tomentosa* leaves are known to have diuretic, hepatoprotective, antioxidant, lipid-lowering, immunomodulatory activities, and protective against acetaminophen-induced mitochondrial dysfunction properties [10, 11].

In this study, we evaluated the influence of the ethanolic extract of *P. tomentosa* on liver-specific serum enzymatic activity associated with chronic ethanol consumption.

MATERIALS AND METHODS:

Plant Material: The leaves of *Premna tomentosa* were collected from in and around Salem, Tamil Nadu, India. The authentication of the plant was done by Dr. S. Sankaranarayana, Head, Department of Medicinal Botany, Govt. Siddha Medical College, Arumbakkam, Chennai with identification voucher no. GSMS/MB- Voucher Specimen no. 23/2017.

Preparation of Plant Extract: The leaves were washed and shade dried for two weeks, after which it was powdered and stored. The dried powder was extracted sequentially by a hot continuous percolation method using the Soxhlet apparatus for 24 h. The solvent from the extracts was recovered under reduced pressure using a rotary evaporator. The obtained crude solid extracts were then freeze-dried for complete solvent removal.

Animals: The study was approved by the Institutional Animal Ethics Committee of Swamy Vivekanandha College of Pharmacy, India (SVCP/IAEC/PhD/2/02/2018). Thirty-six male Wistar albino rats, weighing 150-200 g were kept under standard conditions of temperature (24 ± 2 °C) as well as humidity (60-70%) in a 12-h light -h dark cycles with standard diet and water *ad libitum*.

Experimental Design: Hepatotoxicity was produced by giving Ethanol (40% 1 ml/100 g bw p.o/per day) for ten days and toxicity produced is confirmed with a zero-day elevation of serum biomarkers of liver function. The duration of the experiment was 45 days. The 36 rats were divided into 6 groups (6 each) as follows;

Group I: Control (1ml/100 gm normal saline, bw p.o).

Group II: Ethanol (40% 1 ml/100 g bw p.o).

Group III: Ethanol + Ethanolic extract of *Premna tomentosa* (500 mg/kg bw p.o).

Group IV: Ethanol + Ethanolic extract of *Premna tomentosa* (750 mg/kg bw p.o).

Group V: Ethanol + Liv 52 (1 ml/100 gmbwp.o).

Group VI: Ethanolic extract of *Premna tomentosa* (500 mg/kg bw p.o).

The blood samples were collected on 0^{th} and 45^{th} day from retro-orbital venous plexus and the serum is prepared through centrifuging at 10,000 rpm for 10 min at 30 °C. Serum biomarkers of Liver function including, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) and Serum Total Bilirubin were measured. ALT and AST were measured to determine the concentration of intracellular hepatic enzymes that have leaked into the circulation and served as a marker of hepatocyte injury. ALP was measured to assess biliary function [12].

Statistical Analysis: Statistical program SPSS 16.0 was used. Data were expressed in terms of Mean ± Standard deviation (SD). One-way ANOVA followed by post hoc Duncan's multiple range test was used. The differences were considered significant at $p<0.05$.

RESULTS: Table 1 depicts the initial and final body weight, body weight gain of control and experimental rats. Rats treated with EEPT alone (Group VI) show statistically significant ($p\leq0.05$) differences in the body weight as compared to that of the control rats. Ethanol treated rats (Group II) showed a decrease in weight gain as compared to the control rats. On supplementation with EEPT (Group III & IV) to ethanol-treated rats, the weight gain improved significantly.

TABLE 1: THE EFFECT OF *PREMNA TOMENTOSA* ETHANOLIC EXTRACT ON BODY WEIGHT AND LIVER WEIGHT ON CHRONIC ALCOHOL FED RATS

Groups	Body Weight (gms)		Liver Weight (g/mg)
	Zero-day	Forty-five days	
Control	157.33 ± 3.88	174.50 ± 12.01	3.36 ± 0.39
Ethanol	167.3 ± 5.95	176.33 ± 8.04*	7.61 ± 0.63***
Ethanol + EEPT (500mg/kg)	161.66 ± 6.68	178 ± 5.32	6.05 ± 0.32**
Ethanol + EEPT (750mg/kg)	167.50 ± 4.23	183.16 ± 2.63*	5.08 ± 0.348
Ethanol + liv 52	169.33 ± 7.22	178.50 ± 7.50	5.33 ± 0.64
Only EEPT (500mg/kg)	163 ± 9.52	184.33 ± 8.64**	4.53 ± 0.35*

Values are Mean ± SD (n=6). Data for the normal animal are considered as baseline data; percentage increases (in paratheses) is calculated with reference to normal control $^{*}p\leq0.05$ versus control group, $^{**}p\leq0.001$ versus control group, $^{***}p\leq0.001$ versus control group

TABLE 2: THE EFFECT OF *PREMNA TOMENTOSA* ETHANOLIC EXTRACT ON SERUM MARKERS OF TISSUE DAMAGE ON CHRONIC ALCOHOL FED RATS

Groups	AST (IU/L)		ALT (IU/L)	
	Zeroday	Forty-five days	Zeroday	Forty five days
Control	63.60 ± 3.14	61.20 ± 2.61	55.6 ± 1.03	56.36 ± 1.68
Ethanol	129.90 ± 3.23***	218.95 ± 2.71***	121.48 ± 1.78***	200.13 ± 8.35***
Ethanol + EEPT (500mg/kg)	132.00 ± 4.26	96.01 ± 3.80***	121.83 ± 2.58	61.03 ± 0.87*
Ethanol + EEPT (750mg/kg)	130.82 ± 2.02	87.56 ± 8.81*	123 ± 3.48	57.1 ± 1.27
Ethanol + liv 52	130.98 ± 2.98	101.03 ± 1.58***	122.13 ± 6.66	62.95 ± 2.08**
Only EEPT (500mg/kg)	66.05 ± 4.33	57.26 ± 1.98	61.28 ± 2.10	52.1 ± 2.46

Values are Mean ± SD (n=6). Data for the normal animal are considered as baseline data; percentage increases (in paratheses) is calculated with reference to normal control *p≤0.05 versus control group, **p≤0.001 versus control group, ***p≤0.001 versus a control group

Effect of EEPT alcohol-induced liver damage in rats with reference to biochemical changes in serum is shown in **Tables 2** and **3**. The toxicity produced by ethanol is manifested by a remarkable increase (p<0.001) in serum AST, ALT and ALP in Group II when compared to the control group. The EEPT treated rats differed from normal control rats by a faintly elevated concentration of AST, ALT and ALP at doses 500 and 750 mg/kg/day. These parameters were found to be statistically significantly different as compared to normal rats. The effect was dose-dependent which indicated activation of the regeneration process.

Table 4 depicts the level of serum total bilirubin of control and experimental rats. Rats treated with EEPT alone (Group VI) show a slight significant difference in the level of serum bilirubin as compared to the control rats. However, total bilirubin levels in ethanol alone fed rats (Group II) were significantly increased as compared to the control rats. Supplementation with EEPT (Group III & IV) to ethanol-fed rats significantly decreased (p≤0.05) the levels of bilirubin as compared to the ethanol alone treated rats.

TABLE 3: THE EFFECT OF *PREMNA TOMENTOSA* ETHANOLIC EXTRACT ON SERUM MARKERS OF TISSUE DAMAGE ON CHRONIC ALCOHOL FED RATS

Groups	ALP (IU/L)	
	Zero-day	Forty-five days
Control	92.12 ± 1.95	93.16 ± 6.21
Ethanol	160.95 ± 2.05***	220.88 ± 5.00***
Ethanol + EEPT (500mg/kg)	161.8 ± 2.99	100.8 ± 3.06**
Ethanol + EEPT (750mg/kg)	161.31 ± 3.88	90.85 ± 5.04*
Ethanol + liv 52	167.01 ± 7.56	109.11 ± 4.76**
Only EEPT (500mg/kg)	88.5 ± 3.62	73.45 ± 3.45*

Values are Mean ± SD (n=6). Data for normal animal are considered as base line data; percentage increases (in paratheses) is calculated with reference to normal control *p≤0.05 versus control group, **p≤0.001 versus control group, ***p≤0.001 versus control group

TABLE 4: EFFECT OF EEPT AND ETHANOL ON BILIRUBIN OF CONTROL AND EXPERIMENTAL RATS

Groups	Total Bilirubin (mg/dl)	
	Day 1	Day 45
Control	0.43 ± 0.15	0.53 ± 0.08
Ethanol	1.11 ± 0.17***	1.83 ± 0.08***
Ethanol + EEPT (500mg/kg)	1.16 ± 0.18	0.57 ± 0.07
Ethanol + EEPT (750mg/kg)	1.12 ± 0.22	0.55 ± 0.04
Ethanol + liv 52	1.15 ± 0.18	0.58 ± 0.07
Only EEPT (500mg/kg)	0.53 ± 0.17	0.45 ± 0.08*

Values are Mean ± SD (n=6). Data for normal animal are considered as base line data; percentage increases (in paratheses) is calculated with reference to normal control *p≤0.05 versus control group, **p≤0.001 versus control group, ***p≤0.001 versus control group

DISCUSSION: Oxidative stress is one major factor in the etiology of ethanol injury, mainly by Kupffer cell-derived ROS. Ethanol activates Kupffer cells primarily through the action of a substance called endotoxin, which is released by certain gram-negative bacteria present in the intestine. Kupffer cell activation generates ROS and pro-inflammatory TNF-α, it can lead to liver damage [13]. Growing evidence support the hypothesis that ethanol-induced tissue damage may not only be a consequence of oxidative stress but also due to nutritional deficiencies. Since bodyweight gain by rats supplemented with EEPT suggests the beneficial effects of EEPT against alcohol-induced liver damage [14].

As shown in **Table 2** and **3** ethanol-treated rats increased in serum activities compared to normal control rats. Typically, ALT and AST are used to assess liver function; ALT is highly precise in the monitoring hepatocellular status, and AST is a sensitive indicator of mitochondrial problems, particularly in centrilobular areas of the liver [15]. An increase in the liver enzymes may attribute to the damaged structural integrity of the liver, which

results in the leakage of these enzymes from the cytosol into the bloodstream [16]. Total protein, bilirubin and ALP levels are correlated to the liver function of hepatocyte [17]. Bilirubin and ALP levels are the most useful clinical clues to the severity of necrosis, biliary pressure and its accumulation is a measure of binding, conjugation and excretory capacity. Any abnormal increase of total bilirubin traduces hepatobiliary disease and severe disturbance of hepatocellular architecture while an increase in ALP is due to increase synthesis; in the presence of increasing biliary pressure [18]. In our study administration of fraction at the dose of 500 and 750 mg/kg significantly decreased the ALT, AST, ALP and Total Bilirubin towards its normal, indicating EEPT preserved the liver cell damage caused by chronic ethanol consumption.

CONCLUSION: To conclude, the findings confirm that ethanol-induced liver toxicity can be alleviated by the potential beneficial effects of EEPT as evidenced by the improvement in the liver function. The effect was more pronounced in the rats administered EEPT (750 mg/kg b.w) to ethanol-fed rats. EEPT shows significantly reducing elevated serum biomarker levels in the body and shows amelirate activity against ethanol-induced toxicity.

ACKNOWLEDGEMENT: The authors are thankful to principal and management, Vivekanandha Dental College for Women for providing the necessary facilities to carry out this research work.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Ighodaro OM and Omole JO: Ethanol-induced hepatotoxicity in male Wistar rats: Effects of aqueous leaf extract of *Ocimum gratissimum*. Journal of Medicine and Medical Science 2012; 3(8): 499-05.
2. Shanmugam KR, Ramakrishna CH, Mallikarjuna K and Reddy KS: Protective effect of ginger against alcohol-induced renal damage and antioxidant enzymes in male albino rats. Indian Journal of Experimental Biology 2010; 48: 143-49.
3. Balakrishna V and Lakshmi T: Hepatoprotective activity of ethanolic extract of *Terminalia chebula* fruit against ethanol induced hepatotoxicity in rats. Asian Journal of Pharmaceutical and Clinical Res 2017; 10(11): 55-58.
4. Vetriselvan S and Anil M: Chronic ethanol consumption-Induced hepatotoxicity and protective effect of *Boswellia serrata*. National Journal of Physiology, Pharmacy and Pharmacology 2016; 6: 170-73.
5. Seth D, Hogg PJ, Gorrell MD, McCaughan GW and Haber PS: Direct effects of alcohol on hepatic fibrinolytic balance: implications for alcoholic liver disease. J Hepatol 2008; 48: 614-27.
6. Elias A and Bonsome B: Ethanolic leaf extract of *Ocimum gratissimum* ameliorates methotrexate-induced nephrotoxicity in albino rats. Asian J of Med Sci 2018; 9(5): 37-43.
7. Zodape GV and Bhise PP: Effect of *Aloe vera* extract and isoniazid –rifamicin drug on liver histological studies of male Wistar rats. International Journal of Pharmaceutical Sciences and Research 2018; 9(10): 4318-25.
8. Anon. Formulary of siddha medicines, IMPCOPS Publisher 2018, Chennai 1989; 398-02.
9. Pawar SD, Cyriac KS, Meena N, Shirolkar AR, Gaidhani SN and Veluchamy G: Evaluation of antioxidant and hepatoprotective potential of *Premna tomentosa* L: In Albino Wistar Rats. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2016; 7(4): 2990-98.
10. Chin W, Jones WP, Mi Q, Rachman I, Riswan S, Kardono LBS, Chai HB, Fransworth NR, Cordell GA and Swanson SM: Cytotoxic clerodane diterpenoids from the leaves of *Premna tomentosa*. Phytochemistry 2006; 67: 1243-8.
11. Banu MJ, Elavarasan G, Devi JA and Muthu AK: *In-vitro* antioxidant activity of various extracts of aerial parts of *Premna tomentosa*. International Journal of Chemical and Pharmaceutical Sciences 2015; 6(2): 21-26
12. Sathiya S, Christilda F, Sundarapandian S and Ponmozhi S: Hepatoprotective activity of *Musa sapientum* fruit peel (MSPE) against streptozotocin – induced toxicity in rats. International Journal of Pharmacy 2015; 5(1): 165-69.
13. Hoek JB and Pastorino JG: Ethanol, oxidative stress and cytokine-induced liver cell injury Alcohol 2002; 27: 63-68.
14. Vijay M, Aktarul IS, Sivaranjani A and Nisha S: Zingerone ameliorates hepatic and renal damage in alcohol-induced toxicity in experimental rats. International Journal of Nutrition, Pharmacology, Neurological Diseases 2019; 6(3): 125-32.
15. Chiu YJ, Chou SC and Chiu CS: Hepatoprotective effect of the ethanol extract of *Polygonum orientale* on carbon tertrachloride-induced acute liver injury in mice. Journal of Food and Drug Analysis 2018; 26: 369-79.
16. Monika S, Pranay, Anikita and Shubhangi: Hepatoprotective evaluation of *Bixa orellana* L. seed extract against acute ethanol-induced hepatotoxicity in rats. IJPSR 2018; 9(12): 5168-76.
17. Mutiat AO, Oyinlade CO and Adeteju OL: Hepatoprotective effect of *Mangifera indica* stem bark extracts on paracetamol-induced oxidative stress in albino rats. Eur Sci J 2015; 11: 299-09.
18. Monira A and Naima ZM: Evaluation of protective and anioxidant activity of Thyme (*Thumus vulgaris*) extract on paracetamol-induced toxicity in rats. Aust J Basic Appl Sci 2012; 6: 467-74.

How to cite this article:
Gomathipriyadharsini T, Kavimani M, Sathiyanarayanamurthy S and Prabhu K: Ethanolic leaf extract of *Premna tomentosa* ameliorates alcohol induced hepatotoxicity in albino rats. Int J Pharm Sci & Res 2020; 11(5): 2411-14. doi: 10.13040/IJPSR.0975-8232.11(5).2411-14.