International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Preventive Effect of Alcoholic Extract of *Eugenia Jambolana* Seed on Dexamethasone Induced Hepatic Steatosis in Rats

Sarath Babu. K[1], Nagendra Nayak[2], Hebbal. G.V[3]

[1]Assistant Professor, Department of Pharmacology, Sree Mookambika Institute of Medical Sciences, Kulasekram, Kanyakumari, Tamil Nadu.
[2]Professor, Department of Pharmacology, K.S. Hegde Medical Academy, Nitte University, Manglore, Karmataka.
[3]Professor, Department of Anatomy, Sree Mookambika Institute of Medical Sciences, Kulasekram, Kanyakumari, Tamil Nadu, India.

Corresponding Author: Sarath Babu. K

Received: 20/10/2014 *Revised: 18/11/2014* *Accepted: 02/12/2014*

ABSTRACT

High dose of dexamethasone administration can cause development of non-alcoholic fatty liver. This study was conducted to screen anti-steatosis effect of *Eugenia jambolana (E.J)* with comparison to standard drugs. Wister Albino rats weighing between 230-250 gm were selected and divided in seven groups with each group consisting of 6 rats. G-I: Control (Normal Saline), G-II: Dexamethasone (4 mg/kg/i.p), G-III: Rosiglitazone (16 mg/kg/orally), G-IV: Metformin (1 g/kg/orally), G-V: *E.J* (3 gm/kg/orally), G-VI: *E.J* (6 gm/kg/orally), G-VII: *E.J* (12 gm/kg/orally). *E.J* extract and standard drugs were administered to rats, 6 days before and 6 days during dexamethasone administration. On 12th day rats were scarified under anesthesia and the liver was subjected for histopathological observation. Dexamethasone administered groups showed fatty changes in liver. Pre-administration of drugs and extract prevented the fatty changes in liver. High dose *E.J* extract administration showed the effect like standard drugs. *E.J* seed extract can be used to prevent or treat drug induced non-alcoholic fatty liver with fewer side effects.

Keywords: Dexamethasone, *Eugenia jambolana,* Metformin, Non-alcohol fatty liver, Rosiglitazone, Steatosis,

INTRODUCTION

Liver plays a major role in the metabolism of drugs and environmental toxins. Microsomal enzyme systems (Cytochrome P450) in the liver are affected by fasting, high lipid diet, liver diseases (Fatty liver, hepatic cancers, fibrosis and necrosis) diabetes and drugs.[1] Polymorphism in the enzyme system leads to decrease or increase the metabolism of drugs and other materials. Drugs like steroids cause development of insulin resistance that leads to type 2 diabetes mellitus. Uncontrolled diabetes stimulates the hepatic enzyme expression.[2] Due to insulin resistance the glucose is not utilized by the body cells which lead to hyperglycemia. These stimulate the liver enzymes for synthesis of fatty acids which leads to hyperlipidemia. Increased serum free fatty acids causes development of hepatic steatosis.[3] It possesses histological signs of fibrosis, fat vacuoles and enlargement of hepatocytes. Administration

of dexamethasone elevates the expression of hepatic enzymes which will changes to the pathogenesis of fatty liver.[4] Drugs decreasing glucose and lipid levels are prescribed in the treatment of fatty liver. Use of synthetic drugs can cause development of adverse effects. Natural products are the alternative choice to treat nonalcoholic fatty liver with fewer side effects. *Eugenia jambolana (E.J)* seed powder used in the treatment of diabetes mellitus, hyperlipidemia, to reduce inflammation, central nervous system disorders, infections and diarrhea.[5-11] The present study was conducted to evaluate the alcoholic extract of *E.J* seed powder against dexamethasone induced hepatic steatosis in rats.

MATERIALS AND METHODS

Animal care and handling

All the rats were maintained according to guidelines of National Science Academy, New Delhi, India. Healthy Wistar Albino (230-250 gm), male rats from the central animal house of Sree Mookambika Institute of Medical Sciences, Kulasekaram, Kanyakumari (Dist), Tamil Nadu was selected for the study. They were housed under aseptic controlled conditions for room temperature with 50% humidity and 12 h-12 h of light and dark cycle. Each rat in all the groups was housed individually in polypropylene cages containing paddy husk as bedding throughout the study period. Rats were allowed free access to food and water.[12] The study was planned and conducted after obtaining the approval from Institutional Animal Ethics Committee (Nitte University, Mangalore, Karnataka and Sree Mookambika Institute of Medical Sciences, Kulasekharam, Kanyakumari, Tamil Nadu).

Collection of E.J seeds and preparation of alcohol extract

E.J seeds were collected in the month of June (Kanyakumari, Tamil Nadu). Seeds were dried at room temperature and grounded in electronic grinder to have fine coarse powder. The seed powder (4 kg) was extracted with alcohol in a Soxhlet apparatus. The extract was concentrated by keeping in water bath at 40^0C till all the solvent had completely evaporated from mixture.[13,14] The yield of 10% concentrated extract was stored and used for study.

Study design

Group-I: Control (Normal Saline)
Group-II: Dexamethasone (4 mg/kg/i.p)[15]
Group-III: Rosiglitazone (8 mg/kg/orally) + Dexamethasone (4 mg/kg/i.p)[16]
Group-IV: Metformin (500 mg/kg/orally) + Dexamethasone (4 mg/kg/i.p) [17]
Group-V: Alcoholic extract of *E.J* (3 gm/kg/orally) + Dexamethasone (4 mg/kg/i.p)
Group-VI: Alcoholic extract of *E.J* (6 gm/kg/orally) + Dexamethasone (4 mg/kg/i.p)
Group-VII: Alcoholic extract of *E.J* (12 gm/kg/orally) + Dexamethasone (4 mg/kg/i.p)[18]

Drugs administration

All the drugs were administered to their respective groups except control group 1 to 6 days alone and 7^{th} day to 12^{th} day along with dexamethasone.

Procedure

Standard and test drugs were suspended in 2% gum acacia and administered to the respective groups. All the group of animals were kept for fasting over night on 12^{th} day. Rats were scarified under anesthesia. The liver was perfused with 10% formalin solution to drain blood and other materials. Liver weights and volumes were measured and stored in 10% formalin solution and subjected to H&E stain. The slides were prepared by standard histopathology procedure. All the slides were observed under microscope. [19]

Statistical analysis

The data were expressed as mean and standard error of mean. SPSS (20.0) version software was used for statistical analysis. ANOVA followed by Dunnet t (Post hoc test) was used to find the statistical significance between the groups. P value of less than 0.05 was considered as statistical significant at 95% confidence interval.[20]

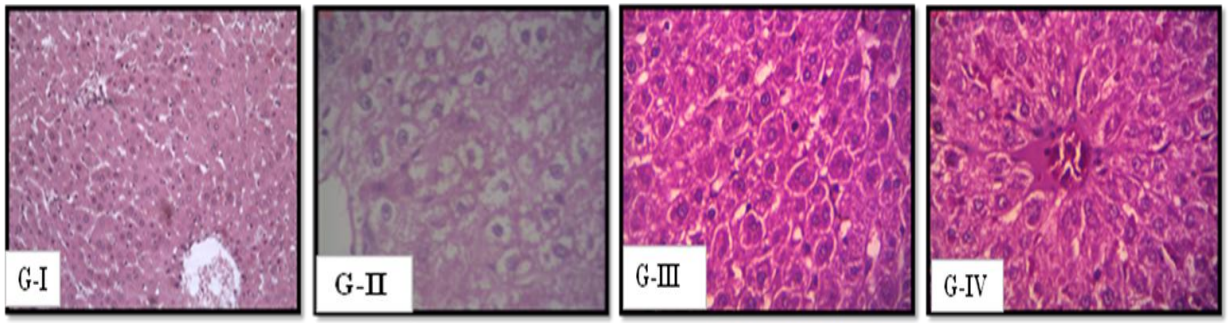
RESULTS

Control group livers showed normal hepatic lobules. Each lobule showed normally arranged hepatocytes forming cords around the central vein. Hepatocytes appeared polygonal in shape with nuclei. Examination of liver sections of rats administered dexamethasone showed enlargement of hepatocytes reaching to ballooning. Cells in the hepatic lobule were seen to contain fat deposition, macro vacuoles deposited all over the cytoplasm and nucleus pushed to one side. Administration of alcoholic extract of *E.J* seed powder significantly prevented the dexamethaosne induced hepatic steatosis. Significant anti-steatotic effect of plant extract was observed at 12 gm/kg administered groups. Metformin and rosiglitazone also significantly prevented the dexamethasone induced fatty liver (Fig.1, 2). Livers of dexamethasone groups showed significant difference in weight and volume compared to control group. Plant extract and standard insulin sensitizer drugs significantly decreased the liver weights and volumes compared to dexamethasone group (Table-1).

Table-1: Comparison of liver weight (gm) and volume (ml) between the groups

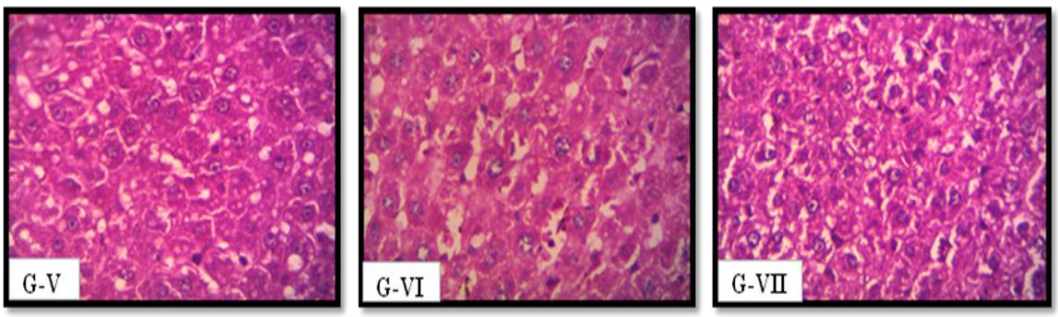
Groups	Liver weight(gm) (MEAN±SEM)	Liver volume (ml) (MEAN±SEM)
Group-I	3.63±0.64	2.63±0.12
Group-II	10.75±0.10*	11.85±0.13*
Group-III	6.86±0.54*,#	5.95±0.84*,#
Group-IV	6.12±0.98*,#	5.23±0.12*,#
Group-V	7.28±0.89*,#,$,‖	7.98±0.45*,#,$,‖
Group-VI	6.84±1.67*,#	7.05±0.56*,#,$
Group-VII	4.56±1.04*,#,$,‖,¶, ǂ	4.01±0.67*,#,$,‖,¶,ǂ

(*P value significant compared group-I with other groups, #P value significant compared group-II with other groups, $P value significant compared group-III with other groups, ‖P value significant compared group-IV with other groups, ¶P value significant compared group-V compared with other groups, ǂ P value significant compared group-VI with other groups)



(G-I: Control, G-II: Dexamethsone, G-III: Rosiglitazone, G-IV: Metformin)

Figure-1: Effect of Rosiglitazone and Metformin on dexamethasone induced hepatic steatosis in rats.



(G-V: Alcohol extract of *E.J* (3gm/kg), G-VI: Alcohol extract of *E.J* (6gm/kg), G-VII: Alcohol extract of *E.J* (12gm/kg)

Figure-2: Effect of alcohol extract of *Eugenia Jambolana* seed powder on dexamethasone induced hepatic steatosis in rats.

DISCUSSION

Dexamethasone is long acting synthetic steroid. It causes metabolic disorders and morphological adverse effects on several organs of the body such as liver, kidney, bone, eye and testes etc. Most important effect of dexamethasone is insulin resistance. Long term hyperglycemia stimulates the hepatic enzymes leading to increase in serum triglyceride levels. These triglycerides will be deposited in the hepatocytes causing non alcoholic fatty liver. This condition is associated with higher mortality and increased risk of liver related death and cardiovascular disease. It should be diagnosed at the starting stage otherwise, over a time period it leads to liver cirrhosis. Once cirrhosis develops, there will be an increased risk of liver cancer. The present study was conducted to evaluate the anti-steatotic effect of alcoholic extract of *E.J* seed powder against dexamethasone induced hepatic steatosis in rats. Study results showed that dexamethasone administration lead to increased liver weight, volume and fat deposition compared to control, standard and test drug groups. Prior administration of *E.J* seed extract prevented the dexamethasone induced hepatic changes in rat. Anti-steatotic effect of alcoholic extract of *E.J* seed (12 gm/kg) was equivalent to the standard oral hypoglycemic drugs.

CONCLUSION

Alcoholic extract of *E.J* seed powder decreased the liver volumes, weights and significantly prevented the dexamethasone induced steatosis in rats. This study suggests that *E.J* seed powder can be used in the treatment of non alcoholic fatty liver in future.

REFERENCES

1. Woodcroft KJ, Novak RF. Insulin differentially affects xenobiotic-enhanced cytochromre P-450 (CYP) 2E1, CYP2B, CYP3A, and CYP4A expression in primary cultured rat hepatocytes. J Pharmacol Exp Ther 1999; 289: 1121-1127.
2. Weltman MD, Farrell GC, Hall P, Ingelman SM, Liddle C. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic statohepatitis. Hepatology 1998; 27: 128-133.
3. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroentrology 2002; 123: 134-140.
4. Leclercq IA, Farrell GC, Field J, Bell DR, Gonzalez FJ, Robertson GR. CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic statohepatitis. J Clin Investig 2000; 105: 1067-1075.
5. Chopra RN, Chopra IC, Handa KL, Kapur LD, Indigenous drugs of India, 2nd ed, Dhar and Sons, Calcutta 1958:686–689.
6. Grover JK, Vats V, Rathi SS. Anti-hyperglycemic effect of *Eugenia jambolana* and *Tnospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. J Ethnopharmacol 2000; 73(3): 461-470.
7. Chaudhuri N, Pal AK, Gomes S, Bhattacharya A. Anti-inflammatory and related action of *Syzygium cumini* seed extract. Phytother Res 1990; 4(1): 5–10.
8. Chakrabarty D, Mahapatra PK, Chaudhuri AKN. A Neuro-psycopharmacological study of *Syzygium cumini*. Planta Medic 1985; 2: 139–143.
9. Bhuiyan MA, Mia MY, Rashid MA. Antibacterial principles of the seeds of *Eugenia jambolana*. Bangladesh J Biol 1996; 25: 239–241.
10. Kusumoto IT, Nakabayashi T, Kida H, Miyashira H, Hattari H, Namba T, Shimotohno K. Screening of various plant extracts used in Ayurvedic medicine for inhibitory effects on human immunodeficiency virus type 1 (HIV-I) protease. Phytother Res 1995; 12: 488–493.

11. Hajoori M, Naik M, Naik K, Butani N. Evaluation of antimicrobial activity of *Eugenia jambolana* seed extract against human pathogens. IJPCBS 2013; 3(3): 935-939.
12. Hey KC, Eun KW, Young PJ. Antiobesity effect of *Codonopsis lanceolata* in high calorie/high fat diet induced obese rats. Evidenec Based Complementary and Alternative Medicine 2013: 1-9.
13. Suman BS, Reenu R, Afreena N, Krishna Madhaba Prabhu, Suryaanarayana P. Ameliorative effect of active principle isolated from seeds of *Eugenia jambolana* on carbohydrate metabolism in experimental diabetes. Evidence Based Complementary and Alternative Medicine 2011: 1-9.
14. Dinesh Kumar B, Narasimha Rao Y, Prasad Rao M, Sivasankar R. Anti-rheumatoid arthritic activity of stem bark of *Eugenia jambolana* Olana Lam on complete Freund;s adjuvant induced Rheumatoid Arthritis in Wistar rats. IJPILS 2013; 1(11): 81-98.
15. Rajasekaran S, Jaykar B, Anandan R. Anti-diabetic activity of leaves of *Zizyphus nummularia* by dexamethasone induced diabetic rat model. IJPTR 2013; 5(2): 844-851.
16. Mohanraghupathy S, Jayabharath N, Bhuvana teja Y, Hameera Khanam B, Lavanya Lahari B. Effective hypoglycemic action og metformin combinations against dexamethasone induced diabetes mellitus in rats. IJRPB 2013; 1(3): 401-403.
17. Gaikwad AB, Viswanad B, Ramarao P. PPAR-a agonist partially restores hyperglycemia induced aggravation of vascular dysfunction to angiotensin II in thoracic aorta isolated from rats with insulin resistance. Pharmacol Res 2007; 55: 400-407.
18. Kohli K.R, Singh R.H. *Eugenia jambolana*: a plant drug with potential anti-diabetic properties. Journal of Scientific Research on Plant Medicine 1985; 6: 1–4.
19. Mile J, Kiriakos NA, Melanie JS, Maree AM, Paul AL. The effect of cocoa supplementation on hepatic steatosis, reactive oxygen species and LFABP in a rat model of NASH. Comparative Hepatology 2011; 10(10): 1-13.
20. Ramu R, Naveen Kumar, Sarita K, Vishwanath Swamy KM. Effect of *Echinochloa frumentacea* Link on dexamethasone induced insulin resistance in rats. RJPBCS 2012; 3(3): 1269-1278.

How to cite this article: Sarath BK, Nayak N, Hebbal GV. Preventive effect of alcoholic extract of *eugenia jambolana* seed on dexamethasone induced hepatic steatosis in rats. Int J Health Sci Res. 2015;5(1):151-155.

****