PROTECTIVE EFFECT OF SEA BUCKTHORN SEED OIL EXTRACT ON ACETAMINOPHEN INDUCED HISTOLOGICAL CHANGES OF HEPATOCYTES IN ADULT ALBINO RATS

ANNUM BASIT1, ATTYA SHAHID2, SHAGUFTA NASREEN1

1Department of Anatomy, Postgraduate Medical Institute/Ameer ud Din Medical College, Lahore,
2Department of Anatomy, Rashid Latif Medical College, Lahore.

ABSTRACT

Objective: to reveal the effects of sea buckthorn seed oil on acetaminophen induced variations (Diameter of hepatocytes and vacuolization) in liver of rats.

Methods: it was experimental study, conducted at Post Graduate Medical Institute. This study was performed on 24 adult rats, which were then randomly separated into 4 groups A, B, C and D, the experiment lasted for 13 days. Group A was control received normal saline and distilled water. Group B, received acetaminophen 750mg/kg as single dose on day 10. Group C and D received acetaminophen at dose of 750mg/kg along with sea buckthorn seed oil extract 2.6mg/kg and 5.2mg/kg. Liver was removed and fixed in 10% formalin. To observe the effect of acetaminophen and sea buckthorn seed oil, slides were prepared for histological examination. The diameter of hepatocytes and vacuolization was observed. The evaluation of results was done by using SPSS 21.

Results: In Group B, vacuolization was seen in all animals and significant increase in mean diameter (11.9± 1.5 μm) of hepatocytes was seen. However, in Group C, vacuolization was absent in all animals and mean hepatocyte diameter was (6 ± 0.6 μm). In Group D, no vacuolization was seen in all animals and mean hepatocyte diameter decreased to (5.2± 1.5 μm) after administration of SBT seed oil.

Conclusion: Administration of SBT (2.6mg/kg and 5.2mg/kg) exerts a protective effect against damaging effects of ACM (750 mg/kg) on hepatocytes in albino rats. This hepatoprotective role becomes greater with increasing dose of SBT.

Keywords: ACM: Acetaminophen, SBT: Sea buckthorn seed oil.

How to cite this article: Basit A, Shahid A, Nasreen S. Protective effect of sea buckthorn seed oil extract on acetaminophen induced histological changes of hepatocytes in adult albino rats. Pak Postgrad Med J 2021;32(1): 9-14

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

DOI: https://doi.org/10.51642/ppmj.v32i01.421

INTRODUCTION

Liver is an important vital organ that not only maintains many fundamental metabolic functions but also has prime importance in detoxification and elimination of toxic substances1. Moreover, the increasing rate of morbidity and mortality due to hepatic diseases is directly related to vast number of environmental factors and chemical compounds2.

Hepatotoxicity is caused by conversion of acetaminophen and other toxic agents into highly reactive metabolites with the help of hepatic microsomal system in liver known as cytochrome P4503. Acetaminophen is most extensively used analgesic-antipyretic medicine and leads to centrilobular hepatic necrosis, acute liver failure and death at extortionate doses. High rate of mortality is associated with toxic doses of acetaminophen causing centrilobular liver damage, hepatitis and severe hepatic injury4. Acetaminophen in excessive dose can lead to oxidative stress, hepatocyte degeneration and cell death5. Acetaminophen is considered safe if given at recommended therapeutic dosage, however, in case of overdose it produces harmful effects such as severe hepatic injury, renal necrosis and cardiac failure6,7. Acetaminophen injurious effects are due to formation of its metabolites N-acetyl-p-benzoquinoneimine8. A study on rats revealed that acetaminophen in doses of 250mg/kg and 2g/kg produced changes in liver
histology like hepatocyte vacuolization, portal vein and central vein congestion, pyknosis and inflammatory infiltrate9,13.

Sea buckthorn, a deciduous spiny shrub is found in the temperate zone of Asia and Europe10. In Pakistan, the plant is found in Skardu, Swat and Gilgit11. The generic name “Hippophae” means shining horse and since ancient times it has been used as food for horses that leads to shiny skin12.

It is considered as a rich source of vitamins, minerals, antioxidants and phytochemicals. It is also known as sallow thorn, sea berry, sand thorn and Siberian pineapple13-16. Based on the established benefits and presence of bioactive components11 further research is paramount to explore the advantages of this herb in improving quality of life.

The studies on sea buckthorn berries and seed oil emphasize the therapeutic benefits for example skin disorder, in cancer therapy by apoptosis, anti-inflammatory action by stimulating the immune system etc, as a hepatoprotective factor, treatment of gastrointestinal ulcers, and cardiovascular diseases17-22. Seabuckthorn seed oil contains considerable saturated fatty acids as stearic acid (3-4%), palmitic acid (7-9%) and main unsaturated fatty acids in form of linoleic acid (20-23%), linolenic acid (40-43%), oleic acid (19-22%) and palmitoleic acid (1-3%)23.

The hepatoprotective effects of SBT have been studied with various hepatotoxins such as carbon tetrachloride and ethyl alcohol and results were impressive10,24.

METHODS

Twenty-four adult male albino rats, weighing 170-200g were taken from university of Veterinary and Animal Sciences, Lahore. The rats were handled in accordance with the guidelines for care of experimental animals, as promoted by the Canadian Council of Animal Care. They were kept in cages. They were kept in an aerial area at atmospheric temperature of 28.0±2.0°C and moisture (60±10%) under 12-hour light/dark cycles. They were given standard rat diet and water freely.

After ensuring adaptation for a span of one week, the experiment was started. Twenty-four rats were assigned randomly as 1, 2, 3, 4... Using Stat Trek’s method (Appendix I) they were randomly divided into four equal groups as A, B, C and D, so that each category had 6 rats. Sea buckthorn extract seed oil was obtained from PCSIR, Lahore by Soxhlet extraction technique. Sea buckthorn extract seed oil was obtained from PCSIR, Lahore by Soxhlet extraction technique. Acetaminophen was purchased from Merck Pharmaceuticals, Lahore. ACM and SBT seed oil were dissolved in normal saline and distilled water. Dose was freshly prepared on daily basis and was given through oral gavage and intraperitoneal route. Animals were sacrificed at 13th day and liver was dissected out. Liver was examined for any gross abnormality. It was weighed and then fixed with formalin. Sections were taken from liver. Tissue processing was done. Slides were made after embedding and sectioning, labeled according to the rat number and group and stained with H and E.

Parameters: Hepatocyte diameter (Quantitative) and Hepatocyte vacuolization (Qualitative)

The quantitative data (hepatocyte diameter) was presented in the form of Mean ± S.D (standard deviation) and were assessed by using oculomicrometer. The qualitative data (hepatocyte vacuolization) was presented in the form of frequency and percentages. Anova and Fischer exact test were applied to Quantitative and Qualitative parameters respectively.

RESULTS

After twelve days of experiment all rats remained active. Liver tissue was examined under microscope for hepatocytes diameter and vacuolization.
Figure 1: Bar chart showing comparison of diameter of hepatocytes among groups.

Table 3: Comparison of Hepatocyte Vacuolization among groups.

| Group       | Present | Absent | Total |
|-------------|---------|--------|-------|
| Group A (Control) | 0 (0.0%) | 6 (100.0%) | 6 (100.0%) |
| Group B     | 6 (100.0%) | 0 (0.0%) | 6 (100.0%) |
| Group C     | 0 (0.0%) | 6 (100.0%) | 6 (100.0%) |
| Group D     | 0 (0.0%) | 6 (100.0%) | 6 (100.0%) |

p-value* < 0.001

* Fisher's Exact Test
When diameters of hepatocytes were measured, it was noted that the group A had mean value of 5.6±0.4μm with group B had highest of 11.9±1.5μm. The group C and D had mean diameters of 6±0.6μm and 5.2±0.2μm respectively. The diameter of hepatocyte increased in group B animals and ANOVA showed statistically significant difference among groups with p-value<0.001.

DISCUSSION
Liver damage induced by drugs is the leading cause of acute and chronic liver disease. Most prevalent cause of acute liver failure is acetaminophen induced hepatotoxicity. When taken in therapeutic range it causes least possible side effects while its over dosage leads to hepatotoxicity. Sea buckthorn (Hippophae rhamnoides) an anti-oxidant has been reported to be a wonder medicinal plant. Vitaglione et al., (2004) emphasized that reactive oxygen radicals including superoxide and hydroxyl radicals plays a vital part in hepatic injury and advancement. There is substantial increase in the usage of seabuckthorn as antioxidant, anti-cancerous and anti-inflammatory agent. There is substantial impact with the usage of natural antioxidants because they stimulate the natural defense system which lessens oxidative damage. The reason that led to significant use of herbal medicine in current era includes high price of conventional medicines, adverse drug reactions, and their inefficacy.

Sea buckthorn dramatically decreased the high levels of SGOT, SGPT, and MDA and decreased the levels of reduced glutathione in animals treated with carbon tetrachloride, acetaminophen and ethyl alcohol. This infers that the following herb has hepatoprotective activity.

The liver is the cardinal structure of foremost significance involved in the metabolism and elimination of the medicine. Hepatic injury is regularly linked to centrilobular degeneration, rise in lipid peroxidation and reduction of tissue glutathione levels.

Acetaminophen injurious effects are due to formation of its metabolites N-acetyl-p-benzoquinoneimine. It is then eliminated by immediate conjugation with glutathione as cysteine and mercapturic conjugates. The defensive storage of intracellular glutathione is reduced that leads to hepatic and renal damage.

In the current study, there was significant increase in size of hepatocytes after the use of acetaminophen (p-value<0.001) which was due to swelling of intracellular organs especially mitochondria and endoplasmic reticulum. Hepatic toxicity displays itself in the form of cell vacuolation which is cellular defence mechanism against injurious substances. These substances are segregated in vacuoles and thus prevented from interfering with cellular metabolism. It is inferred that cytoplasmic vacuolation is mainly a consequence of disturbances in lipid inclusions and fat metabolism. This finding correlated with work done by Haouas et al. (2014) in which findings were observed after administration on first day with 750mg/kg acetaminophen disintegrated in normal saline (5ml/kg) intraperitoneally. Similar results were observed in relation to work of (Hsu et al., 2009).
Acetaminophen leads to fatal centrilobular hepatic injury when taken in high dose of 750mg/kg at a single dose on day 10. This is attributed to generation of reactive oxygen and nitrogen varieties in liver cells experiencing destructive variations. The deprived potential of mitochondria to make ATP by augmented oxidative stress leads to mitochondrial permeability transition. The death of cells due to injury and free radical production is caused by depletion of ATP.

**Conclusion**

From the foregoing results, it is clear that seabuckthorn being antioxidant has maintained liver tissue architecture, prevented congestion and significantly reduced degeneration of hepatocytes by counter-balancing acetaminophen induced oxidative stress. Hence it can be recommended as a protective agent against acetaminophen induced liver damage due to its easy, rapid and safe dietary administration especially with the increasing use of acetaminophen as pain killer, anti pyretic and anti inflammatory agent.

Moreover, the contemporary survey will create an understanding for the hazards of the acetaminophen and infer that the individual should lessen the daily usage of acetaminophen for minor ailments.

**ETHICAL APPROVAL**

The study was approved by the Ethical Review Committee of Postgraduate Medical Institute / Ameer-ud-Din Medical College/Lahore General hospital, Lahore via Research No. 00-175-20 Dated: December 08, 2020.

**REFERENCES**

1. Mayuren C, Reddy VV, Priya SV, Devi VA. Protective effect of Livactine against CCl4 and paracetamol induced hepatotoxicity in adult Wistar rats. N Am J Med Sci., 2010 Oct;2(10):491.
2. Zimmerman HJ, Ishak KG. Pathology of the Liver. Hepatic Injury Due to Drugs and Toxins. 2002:622.
3. Rajasekaran A, Periyasamy M. Hepatoprotective effect of ethanolic extract of Trichosanthes lobata on paracetamol-induced liver toxicity in rats. Chinese medicine. 2012;7(1):1-6.
4. Zhu JH, McClung JP, Zhang X, Aregullin M, Chen C, Gonzalez FJ, Kim TW, Lei XG. Comparative impacts of knockouts of two antioxidant enzymes on acetaminophen-induced hepatotoxicity in mice. Exp Biol Med., 2009;234(12):1477-1483.
5. Blough E, Wu M. Acetaminophen: beyond pain and fever-relieving. Frontiers in pharmacology. 2011;9(2):72.
6. Mirochnitchenko O, Weishrot-Lefkowitz M, Reuhl K, Chen L, Yang C, Inouye M. Acetaminophen toxicity: opposite effects of two forms of glutathione peroxidase. J Biol Chem., 1999;274(15):10349-10355.
7. Heard KJ. Acetylcysteine for acetaminophen poisoning. New England Journal of Medicine. 2008;359(3):285-292.
8. Payasi A, Chaudhary M, Singh BM, Gupta A, Sehgal R. Sub-acute toxicity studies of paracetamol infusion in albino wistar rats. JPSDR., 2010;2(2):142-145.
9. Mallhi TH, Qadir MI, Khan YH, Ali M. Hepatoprotective activity of aqueous methanolic extract of Morus nigra against paracetamol-induced hepatotoxicity in mice. Bangladesh J Pharmacol., 2014;9(1):60-66.
10. Hsu YW, Tsai CF, Chen WK, Lu FJ. Protective effects of seabuckthorn (Hippophae rhamnoides L.) seed oil against carbon tetrachloride-induced hepatotoxicity in mice. Food Chem.Toxicol., 2009;47(9):2281-2388.
11. Zeb A. Important therapeutic uses of sea buckthorn (Hippophae): a review. J biol sci., 2004;4(5):687-693.
12. Parmelazhagan T, Chaurasia OP, Ahmed Z. Seabuckthorn: Oil with promising medicinal value. Curr Sci., 2005;88(1):8-9.
13. Hibasami H, Mitani A, Katsuzaki H, Imai K, Yoshioka K, Komiyta Y. Isolation of five types of flavonol from seabuckthorn (Hippophae rhamnoides) and induction of apoptosis by some of the flavonols in human promyelocytic leukemia HL-60 cells. Int J Mol Cell Med., 2005;15(5):805-809.
14. Dhyani D, Maikhuri RK, Rao KS, Kumar L, Purohit VK, Sundriyal M, Saxena KG. Basic nutritional attributes of Hippophae rhamnoides (Seabuckthorn) populations from Uttarakhand Himalaya, India. Curr Sci., 2007;92(8):1148-1152.
15. Shah AH, Ahmed DI, Sabir M, Arif SH, Khaliq IS, Batool FA. Biochemical and nutritional evaluations of sea buckthorn (hippophae rhamnoides L. Spp. Turkestanica) from different locations of Pakistan. Pak. J. Bot., 2007;39(6):2059-2065.
16. Xu YJ, Kaur M, Dhillon RS, Tappia PS, Dhalla NS. Health benefits of sea buckthorn for the prevention of cardiovascular diseases. J Funct Foods., 2011;3(1):2-12.
17. Agrawala PK, Goel HC. Protective effect of RH-3 with special reference to radiation induced micronuclei in mouse bone marrow. NISCAIR May; 2002; 40(5):1-3.
18. Goel HC, Prasad J, Singh S, Sagar RK, Kumar IP, Sinha AK. Radioprotection by a herbal preparation of Hippophae rhamnoides, RH-3, against whole body lethal irradiation in mice. Phytomed., 2002; 9(1):15-25.
19. Xing J, Yang B, Dong Y, Wang B, Wang J, Kallio HP. Effects of sea buckthorn (Hippophae rhamnoides L.) seed and pulp oils on experimental models of gastric ulcer in rats. Fitoterapia. 2002;73(7-8):644-650.
20. Yang B, Kallio H. Composition and physiological effects of sea buckthorn (Hippophae) lipids. Trends Food Sci Technol., 2002;13(5):160-167.
21. Chen Y, Zhong X, Liu T, Ge Z. The study on the effects of the oil from Hippophae rhamnoides in hematopoiesis. Zhong yao cai= Zhongyaocai= J Chin medicin mater., 2003;26(8):572-575.
22. Gao ZL, Gu XH, Cheng FT, Jiang FH. Effect of sea buckthorn on liver fibrosis: a clinical study. World Journal of Gastroenterology: World J Gastroenterol., 2003;9(7):1615-1617.
23. Kumar R, Kumar GP, Chaurasia OP, Shashi Baba Singh. Phytochemical and Pharmacological profile of seabuckthorn oil: A Review. Res J Med Plant., 2011;5(5):491-499.
24. Xu M, Wang N, Zhang W, Wang J. Protective effects of seabuckthorn seed oil against acute alcoholic-induced liver injury in rats. J Food Nutr Res., 2014;2(12):1037-1041.
25. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hyman LS, Reisch JS, Schisht FV, Ostapowicz G, Shakil AO, Lee WM. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatol., 2005;42(6):1364-1372.
26. Vitaglione P, Morisco F, Caporaso N, Fogliano V. Dietary antioxidant compounds and liver health. Crit Rev Food Sci Nutr., 2005;44(7-8):575-586.
27. Kallio H, Yang B, Peippo P, Tahvonen R, Pan R. Triacylglycerols, Glycerophospholipids, tocopherols, and tocotrienols in Berries and Seeds of Two Subspecies (ssp. sinensis and mongolica) of Sea Buckthorn (Hippophae rhamnoides). J Agric Food Chem., 2002;50(10):3004-3009.
28. Marzouk M, Sayed AA, Soliman AM. Hepatoprotective and antioxidant effects of Cichorium endivia L. leaves extract against acetaminophen toxicity in rats. J Med Med Sci., 2011;2(12):1273-1279.
29. Lipkan GM, Ollinyk OA. Hepatoprotective effect of the sea buckthorn-and-pinks oil. Likars' ka sprava., 2000;1(6):96-99.
30. Haouas Z, Sallem A, Zidi I, Hichri H, Mzali I, Mehd M. Hepatotoxic effects of lead acetate in rats: histopathological and cytotoxic studies. J Cytol Histol., 2014;5(5):1-6.
31. Ahmad N, Tahir M, Lone KP. Amelioration of acetaminophen induced hepatotoxicity by methanolic extract of pomegranate peels in rats. J Pak Med Assoc., 2016;66(7):859-863.
32. Oz HS, Chen TS. Green-tea polyphenols downregulate cyclooxygenase and Bcl-2 activity in acetaminophen-induced hepatotoxicity. Dig dis Sci., 2008;53(11):2980-2988.
33. Hinson JA, Roberts DW, James LP. Mechanisms of acetaminophen-induced liver necrosis. Adverse drug reactions. Handbook Exp. Pharmacol, 2010;369-405.

AUTHOR’S CONTRIBUTIONS
AB: Manuscript writing, Data collection
AS: Literature review, statistical analysis
SN: Discussion, Literature research, references