

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

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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 ($11.9 \pm 1.5 \mu\text{m}$) of hepatocytes was seen. However, in Group C, vacuolization was absent in all animals and mean hepatocyte diameter was ($6 \pm 0.6 \mu\text{m}$). In Group D, no vacuolization was seen in all animals and mean hepatocyte diameter decreased to ($5.2 \pm 1.5 \mu\text{m}$) 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

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

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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 failure^{6,7}. 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 infiltrate^{9,3}.

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 shinning 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 hepato protective 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%), linoleic 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 impressive^{10,24}.

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 D) 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.

Table 1: Comparison of diameter of hepatocytes (µm) among groups:

Parameters	Group A (Control)	Group B	Group C	Group D	p-value#
Diameter of Hepatocytes (µm)	5.6 ± 0.4	11.9 ± 1.5	6.0 ± 0.6	5.2 ± 0.2	< 0.001*

#One way ANOVA *p value ≤ 0.05 is considered statistically significant

For multiple comparisons, post hoc Tukey test was used which showed that diameter of hepatocytes in group B was significantly higher as compared to group

A, C and D. However, no significant difference was found in diameter of hepatocytes among A, C and D.

Table 2: Pair wise comparison of diameter of hepatocytes among groups (Multiple Comparison)

S. No.	Group	Group	Mean Difference	Std. Error	p-value
1	A	B	-6.30000*	0.496	< 0.001
		C	-0.36667	0.496	0.880
		D	0.38333	0.496	0.865
2	B	C	5.93333*	0.496	< 0.001
		D	6.68333*	0.496	< 0.001
3	C	D	0.75000	0.496	0.449

*The mean difference is significant at the 0.05 level.

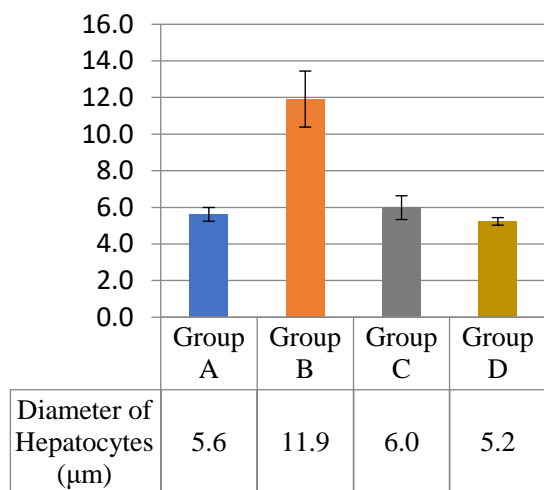


Figure 1: Bar chart showing comparison of diameter of hepatocytes among groups

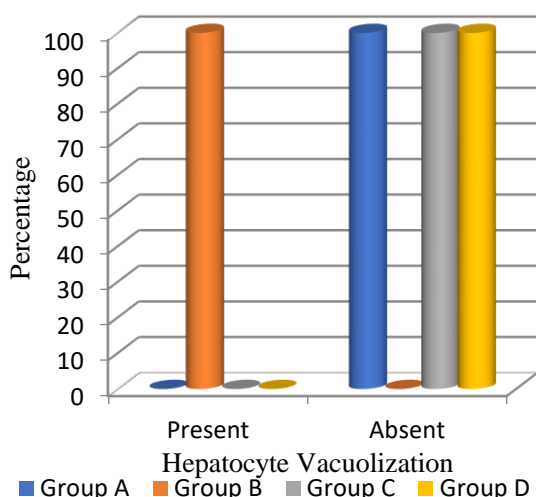


Figure 2: Comparison of Hepatocyte Vacuolization among groups.

It shows that in group A (control) vacuolization was absent. It was present in 6(100%) of group B animals. In group C and D it was present in 4(50%) and 2(25%) of animals.

Table 3: Comparison of Hepatocyte Vacuolization among groups.

	Hepatocyte Vacuolization		Total
	Present	Absent	
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

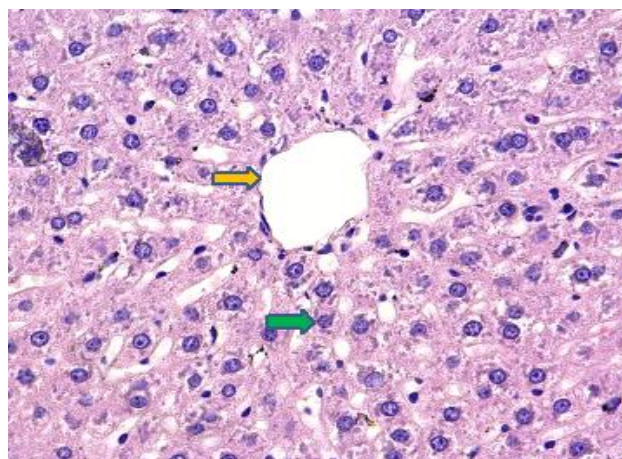


Figure 3: Photomicrograph of liver section from control Group A showing normal central vein (orange arrow), normal hepatocytes (green arrow) at 400X.

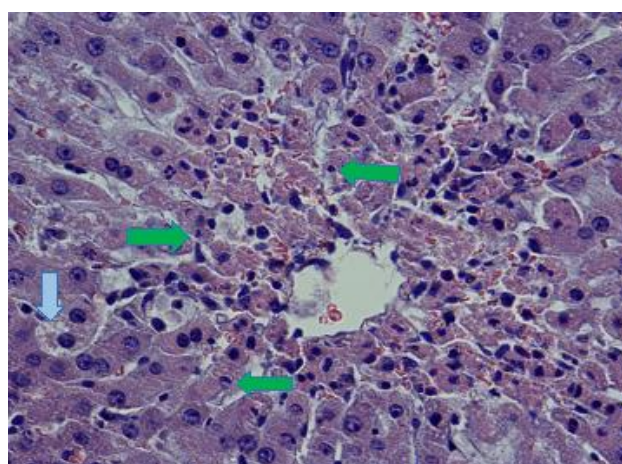


Figure 4: Photomicrograph of liver section from Experimental Group B showing central vein congestion (orange arrow), nuclear pyknosis in hepatocytes (green arrow), hepatocyte vacuolization (light blue arrow) at 400 X.

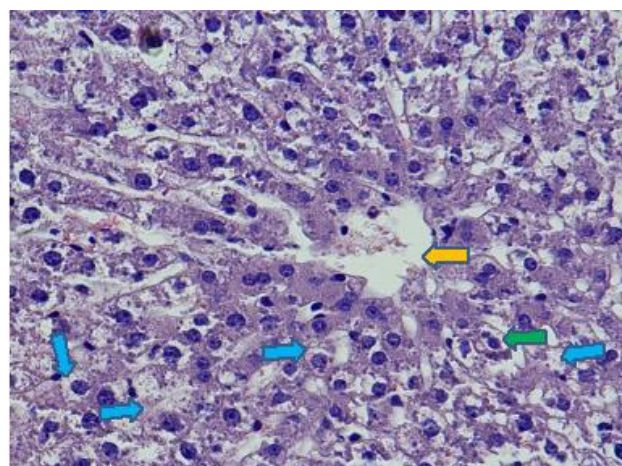


Figure 5: Photomicrograph of liver section from experimental Group B showing central vein congestion (orange arrow), nuclear pyknosis (green arrow), hepatocyte vacuolization (light blue arrow) at 400X.

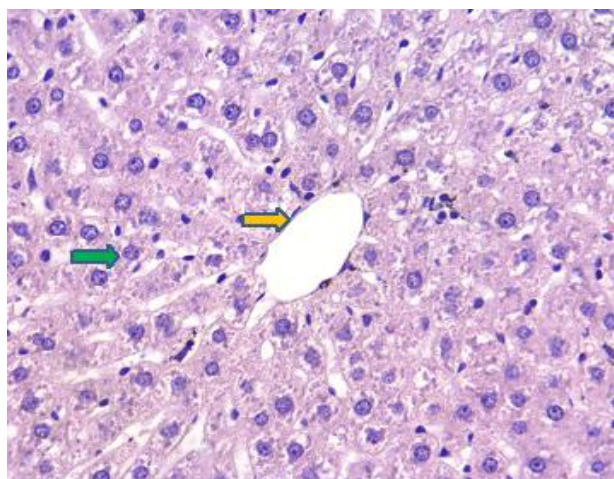


Figure 6: Photomicrograph of liver section from Experimental Group C showing normal looking central vein (orange arrow) and normal hepatocytes (green arrow) at 400X.

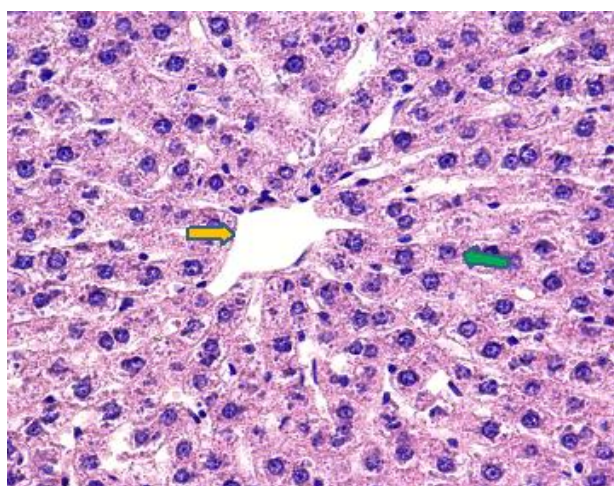


Figure 7: Photomicrograph of liver section from Experimental Group D showing normal looking central vein (orange arrow) and hepatocytes (green arrow) at 400X.

When diameters of hepatocytes were measured, it was noted that the group A had mean value of $5.6 \pm 0.4 \mu\text{m}$ with group B had highest of $11.9 \pm 1.5 \mu\text{m}$. The group C and D had mean diameters of $6 \pm 0.6 \mu\text{m}$ and $5.2 \pm 0.2 \mu\text{m}$ respectively. The diameter of hepatocyte increased in group B animals and ANOVA showed statistically significant difference among groups with $p\text{-value} < 0.001$

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 hepatotoxicity²⁵. Sea buckthorn (*hippophae rhamnoides*) an anti-oxidant, has been reported to be a

wonder medicinal plant²³. Vitaglione et al., (2004)²⁶ 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 agent²⁷. 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 herbual medicine in current era includes high price of conventional medicines, adverse drug reactions, and their inefficacy²⁸.

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 activity²⁹.

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 damage⁸.

In the current study, there was significant increase in size of hepatocytes after the use of acetaminophen ($p\text{-value} < 0.001$) (table 1 fig 1) 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)³⁰.

In the present study, histological examination of the liver preparation manifested that the liver structure was not preserved in Group B (Figure4,5) on account of focal hepatocyte vacuolization, focal central vein congestion and moderate to severe focal nuclear pyknosis. This rearrangement of hepatocytes was previously reported by Ahmed et al. (2016)³¹ 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)¹⁰.

Acetaminophen leads to fatal centrilobular hepatic injury when taken in high dose of 750mg/kg at a single dose on day 10³². 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 ATP³³.

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.

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AUTHOR'S CONTRIBUTIONS

AB: Manuscript writing, Data collection

AS: Literature review, statistical analysis

SN: Discussion, Literature research, references

FF: Data Collection, Conclusion

MYA: Literature Review, Conclusion

MA: Discussion, Literature Review