The immunological and histological effects of the leaves and fruits of the sea buckthorn plant in hepatitis rats.

Lobna Saad Mohammed Abd Elmeged, Billgis Siddig Mohamed Elhag, Iman E. Mohiedeen, Dr. Marwa A. Ahmed

[1a] Department of Nutrition and Food Sciences, Faculty of Home Economics, Menoufia University, Shibin el Kom, Menofia Governorate 6131567, Egypt.
Email : Lobna_lolo_2007@yahoo.com
[1b] Department of Nutrition, Faculty of Applied, AL-Baha University, Saudi Arabia
Email :lobna@bu.edu.sa . ORCID iD: 0000-0003-1527-9457
[2a] Chemistry Department, Faculty of Science, Al –Baha University, Saudi Arabia.
[2b]Chemistry and Biology Department, Faculty of Education , University of Gezira, Sudan
Billgissfaris8@gmail.com ORCID iD :0009-0008-8404-6474
[3] Chemistry Department, Faculty of Science, Al –Baha University, Saudi Arabia.
[4]Department of Food Sciences and Nutrition, College of Sciences, University of Bisha, P.B. 551, Bisha, 61922, Saudi Arabia -Email : marwa@ub.edu.sa

Abstract
Objectives: This study aimed to establish the immunological and histological effects of the leaves and fruits of the sea buckthorn plant on hepatotoxic rats. Materials and Methods: Thirty-six healthy male Sprague Dawley rats were split into six groups, each comprising six mice. a negative control (C -ve) for normal mice. The mice in the remaining groups (number = 30) received injections of CCl_4. All mice were separated into five groups: four groups received BL (5% and 10%) and BF (5% and 10%), while one group served as a positive control (C+ve) that exhibited the sickness but did not adhere to the experimental diet. Results : The maximum RBCs concentration of group's receiving treatment was noted for hepatointoxicated Group"4 " (rats nourished on 10% BL). While the smallest value was noted for Group"5 " (rats nourished on five percent BF) with significant variance (P-value below 0.05). Conversely, the greatest WBCs level of group receiving treatment noted for hepatointoxicated group rats nourished on 5% (BF). While the minimal value noted for hepatointoxicated group rats nourished on 10 % BL) with non-significant distinction (P-value below 0.05) .

Keywords: Immunological effects; hepatitis; sea buckthorn; rats.

Introduction

Animal models are essential for investigating pathways throughout nearly all biological studies. They encompass the whole organism's intricacy and complicate the observation of vivo systems. An in vivo system accurately represents exposure to cellular and profile function, as chemicals are sequentially exposed by absorption at the initial site, then metabolism, distribution, and elimination. Nonetheless, it must mainly use a mechanism like human reactions, and its adverse impact should be clinically significant. Both small animals like mice, guinea pigs, and rabbits and larger species, like monkeys, sheep, pigs, and cattle, are valuable and reliable for investigating the hepatotoxic impacts, distribution, and clearance. They could be used to clarify the basic processes of xenobiotic actions, which will aid in recognizing their effects on human health (Mukherjee et al., 2022).

The applicability of in vivo study results from several animal models to humans could vary because of variations in the metabolism of drugs and pathobiology between species. Given the insufficient evidence to accurately evaluate the efficacy of preclinical animal studies in predicting hepatotoxicity in humans, these investigations may not be adequate as the only modeling systems for hepatotoxicity prediction (Yadav et al., 2021).

The immune system comprises adaptive & innate components. Innate immunity is common in metazoans and is characterized by immunological reactions such as inflammation & phagocytosis. The adaptive component, conversely, involves advanced lymphatic cells able to differentiate between specific "non-self" things and "self" substances. The response to foreign compounds is etymologically termed inflammation, whereas the lack of response to self-materials is called immunity. The two elements of the immune system establish a dynamic biological environment in which "health" is defined as a physical condition whereby the self is immunologically protected while external entities are subjected to inflammatory and immunological eradication. "Disease" may occur when foreign entities cannot be eradicated or self-components are not preserved (Paul & El Bethel Hmar, 2020). In addition to being the biggest solid organ and largest gland, the liver is also one of the most important organs since it acts as a central location for nutrient metabolism and eliminating waste metabolites. A complete failure of liver function may result in fatality in minutes, highlighting the liver's vital importance (Zhou et al., 2024). The primary function is to regulate the safety and transport of chemicals absorbed through the digestive system prior to their distribution into the systemic circulatory system. Sea buckthorn (SB) (Hippophae rhamnoides Linnaeus) is a flowering plant (Angiosperm) classified within the order Rosales and family Elaeagnaceae. SB is characterized physically as a shrub or small tree featuring numerous thorns throughout its structure, and it grows naturally in coastal regions, characteristics which account for its common name. The Latin designation Hippophae rhamnoides originates from ancient Greece. It combines the term "hippo," denoting horse, and "phaos," signifying shine, as equines that consumed foliage from this plant exhibited enhanced coat luster and increased mass (Vilas-Franquesa et al., 2020). Numerous claims on the health advantages of sea buckthorn exist; however, most currently lack effective supporting studies. It appears likely, nevertheless, that it may enhance health in some or all of the following manners. It is worth noting that sea buckthorn is often administered medicinally as an oil extract from seeds, berries, and occasionally leaves. This concentrates its beneficial ingredients; however, it is possible to obtain these by incorporating sea buckthorn berries and juice into your daily diet (Terpou et al., 2019). Sea buckthorn contains numerous minerals, vitamins, antioxidants, and other phytochemicals that may benefit health. Sea buckthorn oil may benefit cardiovascular health by lowering blood pressure, enhancing cholesterol levels, and protecting against clots (Smida et al., 2019). Current and earlier work indicate that sea buckthorn could increase insulin secretion and sensitivity. It may protect against type 2 diabetes; however, further research is required to investigate this issue (Shi et al., 2018). Sea buckthorn oil can help heal sunburns, wounds, frostbite, and bedsores on the skin. It may enhance suppleness and protect against dryness. Sea buckthorn is abundant in advantageous phytochemicals, including antioxidants and flavonoids, which enhance the immune system and could help the body combat illnesses (Rodriguez et al., 2024). The leaves and twigs of sea buckthorn, once considered waste, have been demonstrated to be a useful source of compounds exhibiting significant antioxidant and antibacterial properties (Chen et al., 2020). This plant shows health-promoting qualities, including a beneficial impact on aquaporin expression and bile synthesis and secretion (Parcheta et al., 2021). Sea buckthorn leaves are abundant in phytochemicals, involving phenolic components(like flavonoids and polyphenols),carotenoids, polysaccharides, and saponins. Consequently, SBT leaves exhibit numerous advantageous benefits,including,hepatoprotective,antioxidant, antistress, immunomodulatory, cardioprotective, and antidiabetic properties. In this regard, a recent study by Wei et al., (2022) shows that sea buckthorn leaves represent a promising element due to their nutritional and medicinal properties that enhance the health of both humans and animals. The current study is essential for enhancing this species' exploitation for food, fodder reasons, and medicinal potential. Nevertheless, literature documenting the profiles of numerous bioactive constituents in SBT leaves remains scarce. The need to describe secondary metabolites and nutrients also arises from prior reports on SBT (Woodruff et al., 2024).

Hypothesis

H0: Buckthorn leaves and fruits do not improve the immunological status in hepatitis rats.

HA: Buckthorn leaves and fruits improve the immunological status of hepatitis rats.

1- Materials and methodology

Test substances and chemicals

Buckthorn leaves and fruits have been purchased from a local market in (Jeddah, KSA).

Experimental animals: The researcher used 36 male albino Sprague Dawley mice. Each one of them at the time pf the expertiment weighed 150±10grams.

Used chemicals: Carbon tetrachloride (CCl_4) was obtained as a 10% liquid solution from El-Gomhoryia Company for Chemical Industries, located in Cairo, Egypt. According to Passmore and Eastwood (1986), it was distributed as a liver-toxic chemical agent in white plastic bottles, each with a one-liter capacity.

Methodology

Design

An experimental animal study was conducted over nine days. The rats were split at random across six groups as follows:

1. Group (1): Mice received a basal diet and normal rats as control(-)
2. Group (2): Mice with liver toxicity received a basal diet, as control (+)
3. Group (3): Mice with liver toxicity nourished on a basal diet+ 5%Buckthorn Leaves (BL).
4. Group (4): Mice with liver toxicity nourished on a basal diet of +10 % Buckthorn Leaves (BL).
5. Group (5): Mice with liver toxicity nourished on a basal diet+ 5%Buckthorn Fruits (BF)

6. Group (6): Mice with liver toxicity nourished on a basal diet+ 10% Buckthorn Fruits (BF)

All rats were sacrificed by anesthesia on the ninth day of the experiment. Therefore, the inferior vena cava process has gathered the blood samples in a clean, dry centrifuge tube. Afterward, they were clotted after 20 minutes at room temperature and 15 minutes of centrifugation at 1500 rpm. Moreover, serum samples have been obtained by a dry clean syringe, poured into Wasserman tubes, and frozen at -10 ºC before biochemical analysis. Furthermore, the rat's liver was then eliminated & washed in a saline solution before being dried and weighed. The following formula was used to measure the relative liver weight:

$$\text{Relative liver weight} = \frac{\text{liver organ weight}}{\text{final body weight}} \text{ X } 100$$

Immunological measurements

Determination of HGP, RBC, WBC, and PLT: The concentrations of platelet count (PLT), white blood cell (WBC), red blood cell (RBC), & hemoglobin (HGB) count have been calculated.

Histopathological examination of liver tissue

Tissues were embedded in paraffin after being immersed in a 10% formalin-buffered saline solution, then segmented and stained using hematoxylin and eosin (H&E), as reported by (Carleton, 1979).

Statistical analysis

The data were investigated using a completely randomized factorial design (SAS Users Guide, 1988) upon detecting a significant main effect; the means were separated using the student-Newman-Keuls method. Variations among therapies (P-value equal 0.05 or less) have been deemed significant using the Costas Program. The biological data were examined using one-way ANOVA.

Ethical consideration

The Science Research Ethics Committee of the Faculty of Home Economics accepted the research protocol of this study. They offered the researcher acceptance no #11-SREC-06-2024 to complete this study.

Results

Immunological results: Impact of Buckthorn leaves and Fruits on white blood cells & red blood cells of hepatotoxicity mice.

Red blood cell

Table (1) and figure (1) presents the mean value of red blood cells in hepatotoxic mice subjected to distinct nutrition. The mean value of RBCs in the (C +ve) group has been seen to be lower than that of the control (-) group, measuring 3.10±0.20 and 4.90±0.50 (106/mm3), correspondingly. Hepatotoxic rats fed on various experimental BL (5% and 10%) and BF (5% and 10%) diets exhibited a significant rise in mean values of red blood cells ranging from (3.75 to 4.10(106/mm3). The maximum RBC concentration of cured groups was noted for hepatointoxicated Group 4 (rats nourished on 10% BL). The smallest value observed for Group 5 (5% BF) was significantly different ($P < 0.05$).

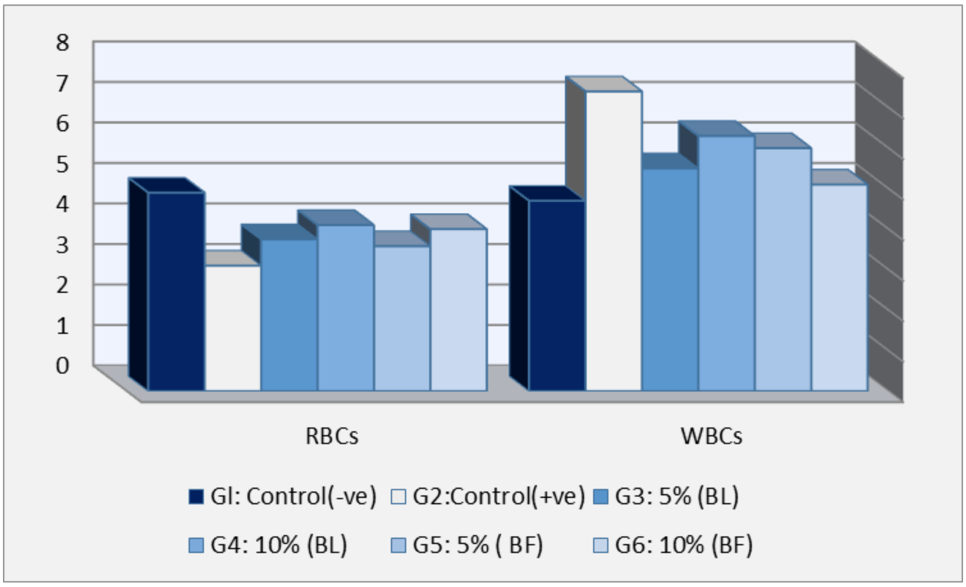
White blood cell

Table (1) and figure (1) presents the mean white blood cell count of hepatotoxic rats subjected to different diets. The mean white blood cell count of the control (+) group was significantly greater compared with the control (-) group, measuring 7.40 ± 0.20 & 4.70 ± 0.30 (10^6/mm³), correspondingly. Hepatotoxic rats subjected to varied experimental BL (five and ten percent) and BF (five and ten percent) diets exhibited a significant rise in mean white blood cell counts, varying from 5.10 to 6.30 (10^6/mm3). The highest amount of red blood cells in the group receiving treatment was observed in hepatointoxicated Group 6 (mice administered a diet containing ten percent BF). The smallest value observed for Group 4 (mice fed on ten percent BL) was significantly different (P <0.05). The results indicated statistically insignificant distinction (P-value below 0.05) among groups 4 and 5 compared to the positive control group.

Table (1): Effect of Buckthorn leaves and Fruits on RBCs and WBCs of hepatotoxicity rats.

Parameters / Groups	RBCs (106/mm3)	W.B.Cs(106/mm3)
Gl: Control(-ve)	4.90^{a}± 0.50	4.70^{c}± 0.30
G2:Control(+ve)	3.10^{b}±0.20	7.40^{a}±0.20
G3: 5% (BL)	3.75^{b}± 0.30	5.50^{b}± 0.30
G4: 10% (BL)	4.10^{a}±0.40	6.30^{b}± 0.40
G5: 5% (BF)	3.58^{b}± 0.60	6.00^{b}± 0.70
G6: 10% (BF)	4.00^{a}± 0.40	5.10^{c}±0.50
LSD:	1.020	1.070

*Values are stated as mean ± S.D.
**Significance is expressed at (p-value below 0.05) utilizing LSD and one-way ANOVA tests.



Figure(1): Effect of Buckthorn leaves and Fruits on RBCs and WBCs of hepatotoxicity rats.

Effect of Buckthorn leaves and Fruits on hemoglobin and platelet of hepatotoxicity mice.

Hemoglobin g/dl

Table (2) and figure (2) below presents the mean hemoglobin concentration in grams per deciliter of hepatotoxic mice subjected to different dietary regimens. The mean hemoglobin value in the control (+) group was 10.20±0.90 grams per deciliter, which was lower than in the control (-) group at 14.24±0.30 grams per deciliter. Hepatotoxic mice administered various experimental BL (five percent and ten percent) and BF (five percent and ten percent) diets exhibited a considerable rise in mean hemoglobin values, ranging from 11.70 to 13.00 grams per deciliter. The greatest hemoglobin level among the group receiving treatment was observed in hepatointoxicated Group 6 (mice administered a diet containing ten percent BF). The lowest value observed for Group 3 (mice fed on five percent BL) exhibited significant variation (P-value below 0.05). The results indicated statistically insignificant distinction ($P<0.05$) among groups 4, 5, and 6 when compared to the (C +ve) group.

Platelet

Table (2) and figure (3) presents the average platelet count of hepatotoxic mice subjected to distinct foods. The mean platelet value of the (C +ve) group was significantly higher than that of the (C -ve) group, measuring 765.0 ± 0.17 compared to 145.0 ± 0.12. Hepatotoxic mice administered varied experimental BL (five & ten percent) and BF (five & ten percent) diets significantly increased mean platelet values, ranging from 152.0 to 173.0. The highest platelet count in the treated groups was observed in hepatointoxicated Group 5 (rats administered a five percent BF diet). The smallest value observed for Group 4 (mice fed on ten percent BL) with significantly different ($P <0.05$).

Table (2): Influence of Bu ckthorn leaves & Fruits on platelet and hemoglobin of hepatotoxicity mice.

Parameters / Groups	Hemoglobin g/dl	Platelet
Gl: Control(-ve)	$14.24^{a} \pm 0.30$	$145.0^{h} \pm 0.12$
G2:Control(+ve)	$10.20^{c} \pm 0.90$	$765.0^{a} \pm 0.17$
G3: 5% (BL)	$11.70^{c} \pm 0.40$	$166.0^{e} \pm .010$
G4: 10% (BL)	$12.93^{b} \pm 0.20$	$152.0^{g} .014$
G5: 5% (BF)	$12.28^{b} \pm 0.60$	$173.0^{d} \pm 0.13$
G6: 10% (BF)	$13.00^{b} \pm 0.40$	$157.0^{f} \pm 0.12$
LSD:	1.010	5.140

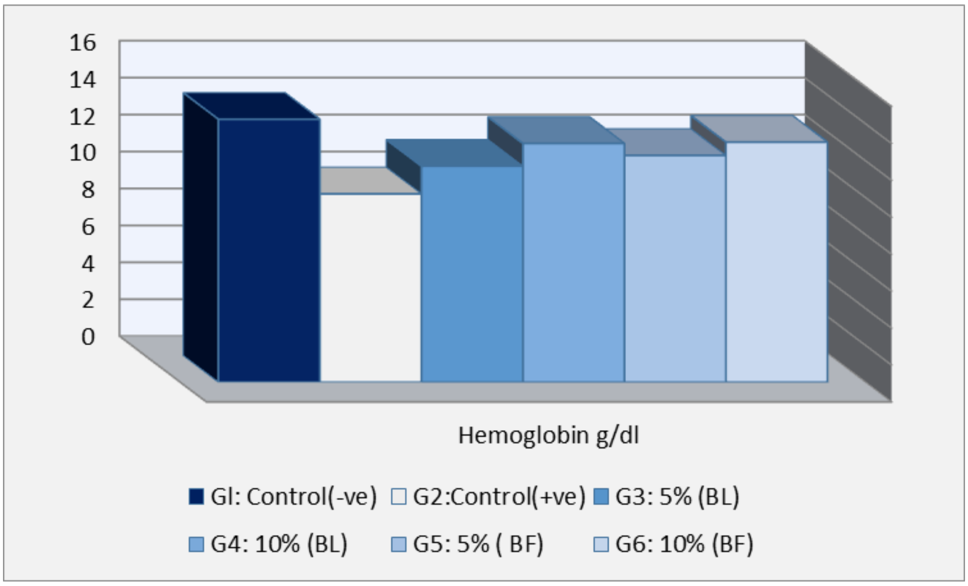
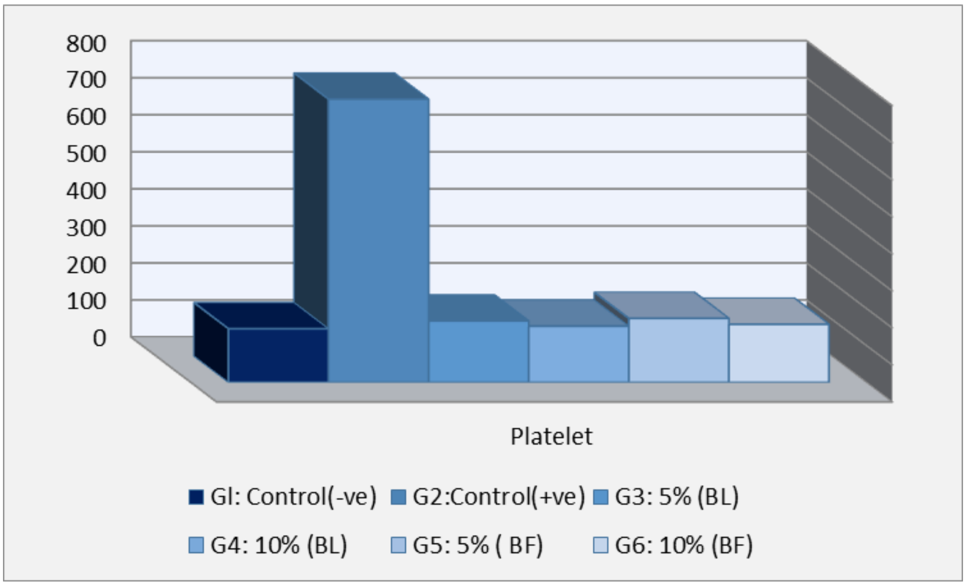


Figure (2): Influence of Buckthorn leaves & Fruits on hemoglobin of hepatotoxicity mice



. Figure (3): Influence of Buckthorn leaves & Fruits on platelet of hepatotoxicity mice

Identification and quantification of phenolic compounds in dried leaves of the sea buckthorn plant by HPLC:

Data presented in table (3) and figure (4) show the identification and quantification of phenolic compounds in dried leaves of the sea buckthorn by HPLC. Data indicated that plant have a high percentage of Chlorogenic acid, Gallic acid, Catechin, Coffeic acid, Methyl gallate, Querectin and Ellagic acid which were 317.79,98.49, 46.61,35.78, 31.70, 22.38 and 11.46 (mg/g) respectively.

Table (3): Identified phenolic compounds in dried leaves of the sea buckthorn plant by HPLC

Phenolic compounds	Dried Saw palmetto seeds (mg/g)
Gallic acid	98.49
Chlorogenic acid	317.79
Catechin	46.61
Methyl gallate	31.70
Coffeic acid	35.78
Syringic acid	11.20
Pyro catechol	ND
Rutin	ND
Ellagic acid	11.46
Coumaric acid	0.82
Vanillin	0.64
Ferulic acid	1.72
Naringenin	ND
Rosmarinic acid	1.50
Daidzein	7.19
Querectin	22.38
Cinnamic acid	ND
Kaempferol	2.07
Hesperetin	3.81

ND=Not detected

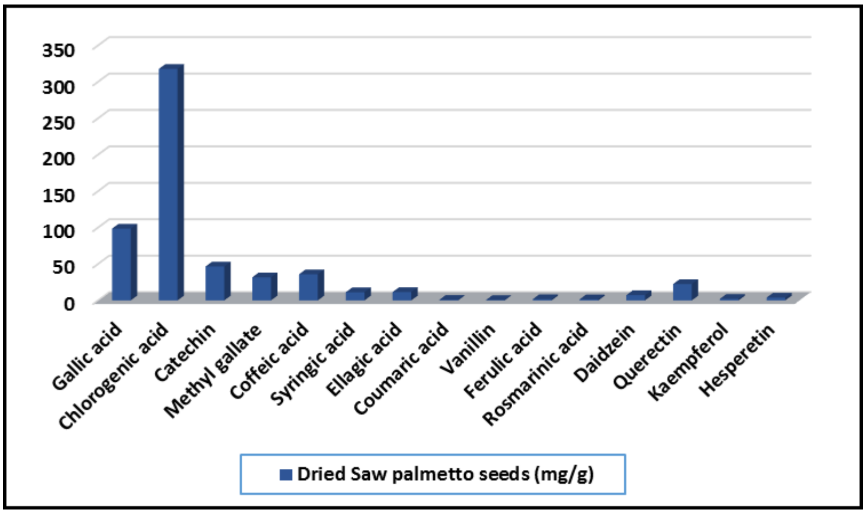


Figure (4): phenolic compounds in dried leaves of the sea buckthorn plant by HPLC

Histopathological results: Histopathological inspection of liver

Rat's liver from group one (C -ve) exhibited the structure of normal histological hepatic lobule (Photos 1). Conversely, the rat's liver from group two (control +) exhibited hepatocellular vacuolar degeneration and congestion of hepatic sinusoids (Photos 2). Some sections investigated from group three (5% BL) showed slight central vein congestion (Photo 3). Meanwhile, sections from group 4 revealed (10% BL) showing hepatocellular vacuolar degeneration (Photo 4). However, the rat's liver from group 5 (5%BF) illustrates slight central hepatic and vein sinusoid congestion (Photo 5). Furthermore, rat's liver from group 6 (10%BF) exhibited slight congestion of hepatic sinusoids & central vein (Photo 6).

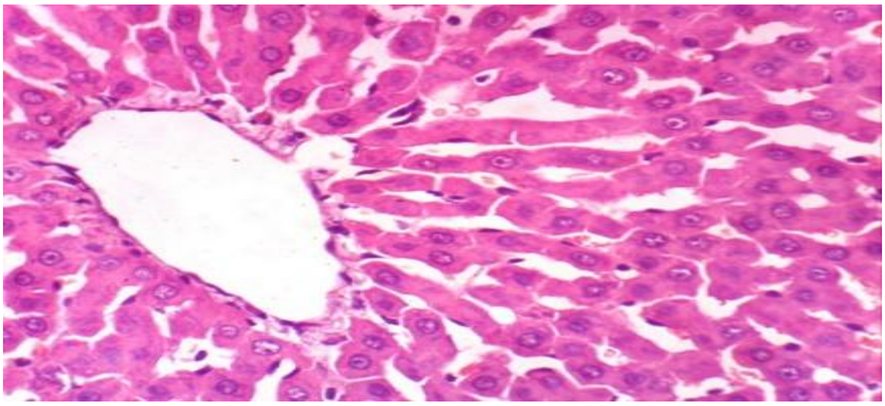


Photo. (1): Mice's liver from group one demonstrating the normal histological architecture of hepatic lobule (Hematoxylin and Eosin X 400).

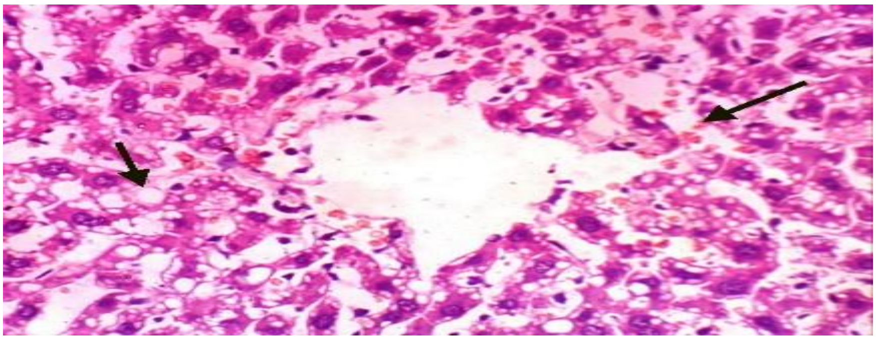


Photo. (2): The mouse's liver from group two demonstrated hepatocellular vacuolar degeneration and congestion of hepatic sinusoids (Hematoxylin and Eosin X 400).

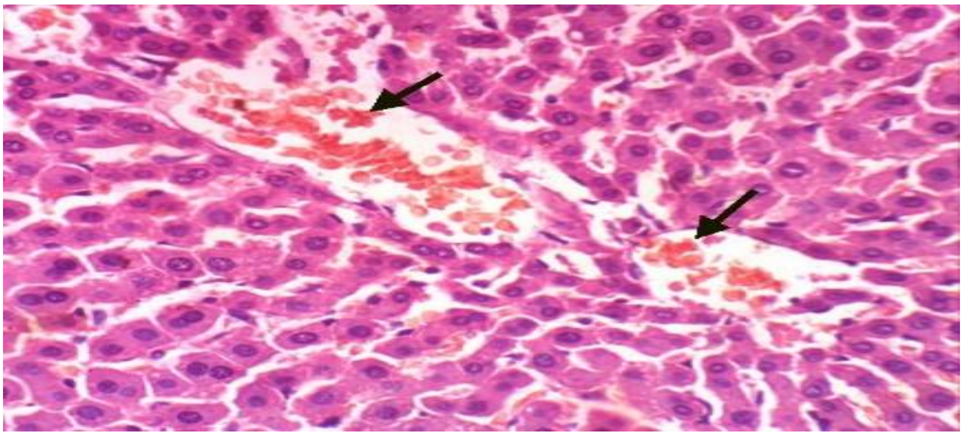


Photo. (3): The mouse's liver from group three demonstrates slight central vein congestion (Hematoxylin and Eosin X 400).

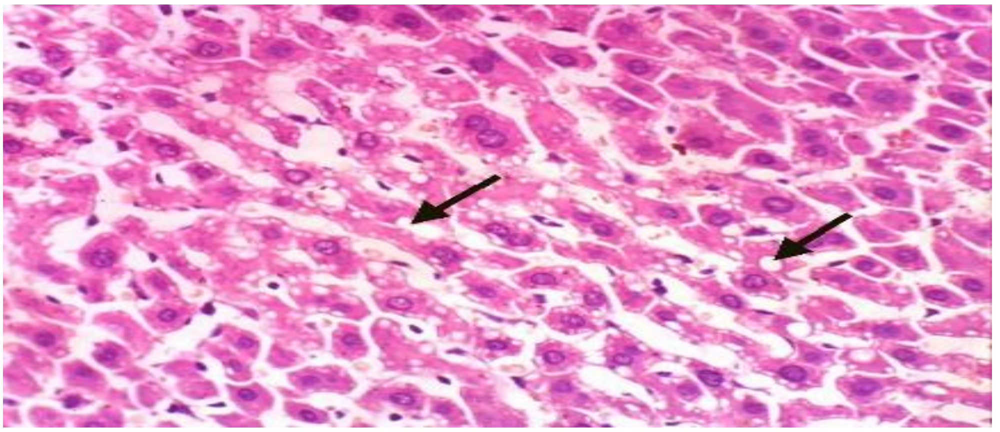


Photo. (4): The mouse's liver from group four demonstrates hepatocellular vacuolar degeneration (Hematoxylin and Eosin X 400).

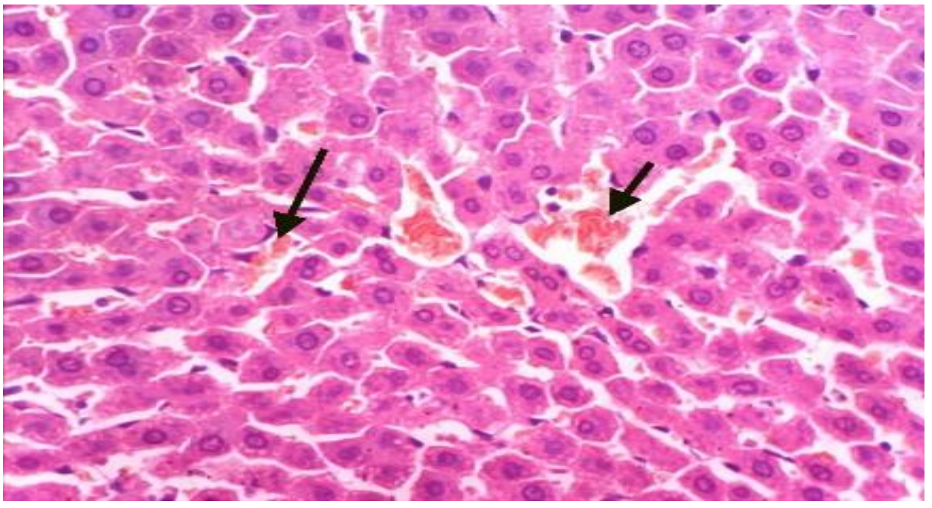


Photo. (5): Mouse's liver from group five demonstrated slight congestion of the central vein and hepatic sinusoids (Hematoxylin and Eosin X 400).

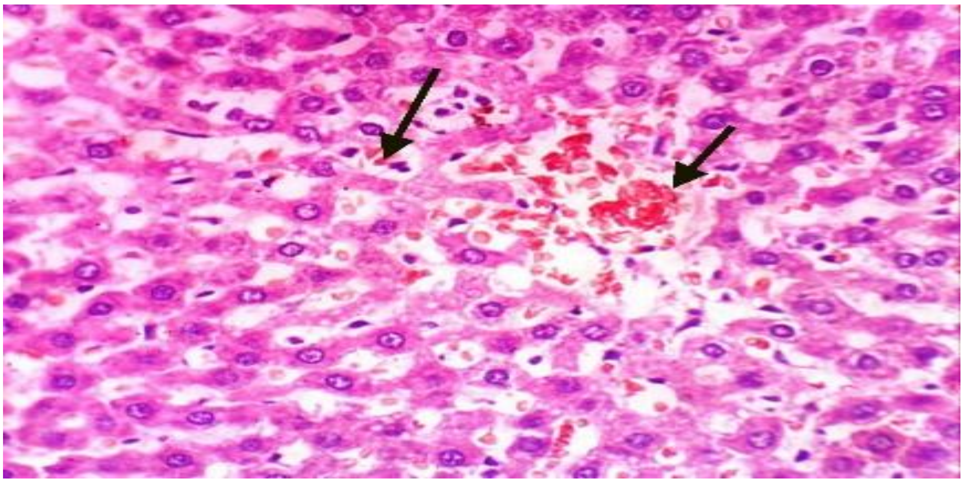


Photo. (6): Mouse's liver from group six demonstrated slight congestion of the central vein & hepatic sinusoids (Hematoxylin and Eosin X 400).

Discussion

Our results showed that the mean value of RBCs in the (C +ve) group has been seen to be lower than that of the control (-) group. Hepatotoxic rats fed on various experimental BL (5% and 10%) and BF (5% and 10%) diets exhibited a significant rise in mean values of red blood cells ranging from (3.75 to 4.10(106/mm3). The maximum RBC concentration of cured groups was noted for hepatointoxicated Group 4 (rats nourished on 10% BL). The smallest value observed for Group 5 (mice nourished a five percent BF diet) exhibited a significant variation (P-value below 0.05). These results align with previous work by Gatlan et al. (2020). Their study showed that sea buckthorn berries are rich in vitamin E, vitamin A, niacin, riboflavin, pantothenic a', vitamin B6, and vitamin B12. Mineral elements contribute to the creation of human tissues, the preservation of proper physiological activities, and the fortification of the immune system. Sea buckthorn berries are rich in minerals like iron, phosphorus, boron, magnesium, aluminum, calcium, and potassium, which contribute to the development of red blood cells.

Our results showed that the mean white blood cell count of the control (+) group was significantly greater compared with the control (-) group. Hepatotoxic rats subjected to varied experimental BL (five and ten percent) and BF (five and ten percent) diets exhibited a significant rise in mean white blood cell counts, varying from 5.10 to 6.30 (10^6/mm3). The highest amount of red blood cells in the group receiving treatment was observed in hepatointoxicated Group 6 (mice administered a diet containing ten percent BF). The smallest value observed for Group 4 (mice fed on ten percent BL) exhibited a significant variance (P-value below 0.05). The results indicated statistically insignificant distinction (P-value below 0.05) among groups 4 and 5 compared to the positive control group. These results correspond with Woodruff et al. (2024), who stated that blood is a vital bodily fluid comprising white blood cells, red blood cells, & platelets suspended in serum at homeostatic concentrations. Blood investigation is an effective method for evaluating the health status of animals, as it is crucial for understanding their nutritional, physiological, and pathological conditions. The evaluation of hematological indicators can indicate the degree of adverse effects on the blood constituents of an animal.

Our results showed that the mean hemoglobin value in the control (+) group was 10.20±0.90 grams per deciliter, which was lower than in the control (-) group at 14.24±0.30 grams per deciliter. Hepatotoxic mice administered various experimental BL (five percent and ten percent) and BF (five percent and ten percent) diets exhibited a considerable rise in mean hemoglobin values, ranging from 11.70 to 13.00 grams per deciliter. The greatest hemoglobin level among the group receiving treatment was observed in hepatointoxicated Group 6 (mice administered a diet containing ten percent BF). The lowest value observed for Group 3 (mice fed on five percent BL) exhibited significant variation (P-value below 0.05). The results indicated statistically insignificant distinction ($P<0.05$) among groups 4, 5, and 6 when compared to the (C +ve) group. The findings align with those of Skalski et al. (2019), who indicated that hemoglobin is a marker for glycemic control and is thus significant in diagnosing diabetes and its consequences. Furthermore, hepatic rats exhibited a twenty-four percent elevation in HbA1c levels relative to the control group. Following treatment with buckthorn extract, HbA1c levels in erythrocytes were assessed.

Our results showed that the mean platelet value of the (C +ve) group was significantly higher than that of the (C -ve) group, measuring 765.0 ± 0.17 compared to 145.0 ± 0.12. Hepatotoxic mice administered varied experimental BL (five & ten percent) and BF (five & ten percent) diets significantly increased mean platelet values, ranging from 152.0 to 173.0. The highest platelet count in the treated groups was observed in hepatointoxicated Group 5 (rats administered a five percent BF diet). The smallest value observed for Group 4 (mice fed on ten percent BL) exhibited a significant variance (P-value below 0.05). These findings are consistent with (Olas et al., 2019). The phenolic-rich fraction of SB fruits has in vitro anti-platelet activity. Moreover, SB leaves and twigs possess phenolic compounds exhibiting antioxidant and anticoagulant properties. The nonpolar fraction derived from SB twigs was shown to extend prothrombin duration and stimulated partial thromboplastin time in the laboratory model; nevertheless, the mechanism underlying the effects of these fractions on the activation of blood platelets remains unidentified. The results are consistent with Skalski et al. (2018), which determined the impact of the phenolic & nonpolar fractions from twigs & leaves of SB on the laboratory blood platelet stimulation. This study precisely investigates the impact of fractions on the adhesion of blood platelets to 2 adhesive proteins, collagen & fibrinogen type one, the predominant collagen type in arteries wall-affected by atherosclerosis. It also explores aggregation of blood platelets triggered by distinct physiological agonists (adenosine diphosphate, collagen, & thrombin), non-enzymatic lipid peroxidation in quiescent platelets, arachidonic a' metabolism (enzymatic lipid peroxidation) in thrombin-activated platelets, & the generation of platelet superoxide anion (O2− radical dot). The investigation examines the laboratory cytotoxicity of the evaluated plant fractions on human blood platelets by assessing the activity of extracellular lactate dehydrogenase.

Table (4): Comparison of Hematological Parameters in Hepatotoxic Rats: Current Study Findings vs. Previous Studies.

Parameter	Current Study Findings	Related Findings from Previous Studies
RBC	Hepatotoxic rats fed with sea buckthorn leaves and fruits showed a significant increase in RBC count compared to the positive control group.	Gatlan et al. (2020): Sea buckthorn berries are rich in vitamins (E, A, niacin, riboflavin, B6, B12) and minerals (iron, phosphorus, magnesium, etc.) that contribute to the development of red blood cells.
WBC	Hepatotoxic rats fed with sea buckthorn diets exhibited a significant increase in WBC count compared to the positive control group.	Woodruff et al. (2024): Hematological indicators, including WBC, are important for assessing health status and the effects of treatments on animals.
Hemoglobin	Hepatotoxic rats fed with sea buckthorn diets showed a significant increase in hemoglobin levels compared to the positive control group.	Skalski et al. (2019): Buckthorn extract affects HbA1c levels in hepatic rats, indicating its impact on blood parameters related to glycemic control.
Platelets	Hepatotoxic rats fed with sea buckthorn diets exhibited a significant increase in platelet count compared to the positive control group.	Olas et al. (2019) and Skalski et al. (2018): Sea buckthorn fractions have anti-platelet activity and affect platelet function, demonstrating its influence on platelet biology.

Conclusion

Sea buckthorn is a distinctive and extremely valuable plant. It spans over two million hectares globally. It also comprises approximately two hundred bioactive components and provides significant health advantages; recent in vitro, in vivo, and clinical investigations conducted over the past five years support the health benefits of SB. These biological actions encompass anticancer, antioxidant, hepatic tissue protection, anti-inflammatory properties, and significant immunological effects. As a multifaceted commercial & ecological plant, SB has a promising future. This ancient herb possesses potent medicinal synergies and has significantly benefited humanity. SB possesses an unparalleled ability to contribute to economic development & enhance the natural environment.

Recommendations

To systematically develop and exploit sea buckthorn resources, researchers need to work on the following:

1. Separation and recognition of further specific bioactive chemicals, together with an in-depth examination of their health-promoting processes.
2. Implementing more clinical investigations to validate the health advantages of SB for humans.
3. Using sea buckthorns for disease treatment & prevention.
4. Formulating functional foods and additional items derived from sea buckthorn.
5. Complying with the principle of integrating economic and ecological considerations and advancing the SB industry scientifically, rationally, and sustainably.

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