WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 4, 1225-1234. Research Article ISSN 2277–7105

BIOCHEMICAL, HAEMATOLOGICAL AND HISTOLOGICAL STUDIES ON AQUEOUS LEAF EXTRACTS OF *SPINACIA OLERACEA*

***Onwusonye Josephat Chukwudi, PhD**

Department of Microbilogy/ Biochemistry, Federal Polytechnic Nekede Owerri, Nigeria.

Article Received on
03 January 2025,

Revised on 22 Jan. 2025,
Accepted on 12 Feb. 2025

DOI: 10.20959/wjpr20254-35598

***Corresponding Author**
Onwusonye Josephat Chukwudi
Department of Microbilogy/ Biochemistry, Federal Polytechnic Nekede Owerri, Nigeria.

ABSTRACT

The present study was conducted to evaluate the effect of aqueous leaf extracts of ***Spinacia oleracea*** on haemoglobin (Hb), Packed cell volume (PCV), blood sugar, serum urea and pancreatic cellular integrity in albino rats, Twenty (20) healthy rats were randomly divided into five groups, 1-5 (n=4). Group 1 served as normal control rats kept on feed and water only while groups 2-5 served as treatment groups that received 200 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg respectively of the extract daily for 3 weeks. Hematological investigations revealed a dose-dependent increase in Hb concentration and PCV, with the highest dose (800 mg/kg) yielding the most significant improvements ($p<0.05$) compared with the control. There were significant reductions in serum urea concentrations and blood sugar levels, both occurring in dose dependent manners. These findings are consistent with previous studies highlighting the role of dietary antioxidants in enhancing red blood cell synthesis and protecting against oxidative stress. Histological examination of the pancreas across all groups showed no adverse effects, with tissue architecture remaining intact and free of inflammation or necrosis. The preservation of pancreatic integrity even at higher doses indicates the extract's safety and potential protective effects on pancreatic tissue. These outcomes align with the known bioactive properties of ***Spinacia oleracea***, including its high content of carotenoids, flavonoids, and phenolic compounds, which mitigate oxidative stress and inflammation, critical factors in pancreatic cellular health. The study underscores the multiple benefits of ***Spinacia oleracea*** as a natural hematopoietic agent, preserving liver health, enhancing erythropoiesis and preserving pancreatic cells. In conclusion, the findings suggest that leaf extracts of ***Spinacia oleracea*** is a safe and effective natural remedy with potential

applications in managing anemia, diabetes and protecting hepatocellular and pancreatic health. This study, therefore contributes to the growing body of evidence supporting the incorporation of ***Spinacia oleracea*** into dietary and medicinal products for improving overall human and animal health.

KEYWORDS: Biochemical, haematological, histological, *spinacia oleracea*.

INTRODUCTION

Plants are very important sources of medicines. The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance. The therapeutic advantages derived from herbal products in disease management include their effectiveness, availability and safety (Dash and Murthy, 2011).

Plant derived products have been used for medicinal purposes for centuries and presently, it is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs. This may be attributable to the down turn in the economy, as herbal medicine is perceived to be a cheaper means of treatment (Woldeyes *et al*., 2012).

Spinach, a plant with the richness in several vitamins and minerals is a very beneficial plant to human health. Increasing exposure to the bioactive compounds by including spinach in regular meals and salads, or by partially swapping spinach for lettuce, would likely result in good health consequences (Okunade, 2012).

Spinach has a high nutritional value and is extremely rich in antioxidants. Apart from having nutritional value, it has been also credited with various biological activities like virus inhibitor, antihelmentic, antioxidant, hepatoprotective (Bosi *et al*., 2013).

Human and animal research have also shown that spinach-derived chemicals can help with obesity, hyperglycemia, and hypertriglyceridemia, all of which are symptoms of the metabolic syndrome. The leaves of spinach are used in various pharmacological activities such as, anti-cancer properties, anti-inflammatory properties, anti-obesity properties etc. Phytochemicals like flavonoids, carotenoids and phenolic groups have been noticed in high amounts in the plant (Kohler and Baghdadi-Sabeti, 2011).

The seeds are useful in fevers, leucorrhoea, and other notable human ailments. Spinach is used for urinary calculi (Khare, 2017)

Spinacia oleracea known for its nutritional uses in food processing alongside with its pharmacological properties such as, anti-cancer properties, anti-inflammatory properties, anti-obesity properties etc owing to various phytochemicals in the leaf which includes; flavonoids, carotenoids and phenolic groups etc. However, despite its high nutritional and medicinal usage, limited scientific exploration has been conducted regarding its toxicity on body organs like liver, kidney, pancreas etc. Therefore, the necessity for the present study.

MATERIALS AND METHODS

COLLECTION AND PREPARATION OF SAMPLE

Fresh mature spinach leaves were collected from a garden in Ihiagwa, Imo State and taken to the laboratory for further processing. The fresh leaves were washed with tap water and cut into pieces. Afterwards, they were air dried for two weeks. The dried samples were ground into powder using an electric blender. Five hundred grams of the ground sample was soaked with distilled water in a clean container with a tight cover for 72 hours. The soaked sample was filtered using a filter paper. The filtrate was dried to a slurry in a rotary evaporator to get the crude extract.

EXPERIMENTAL DESIGN

The animal care and handling was done according to the United States National Institute of Health Principles of Laboratory Animals Care (NIH, 1978). Twenty adult male albino rats used for the study were maintained under controlled conditions of temperature, humidity, and light. They were housed in large metal cages.

The rats were allowed to acclimatize for one week prior to treatment. At the start of experiment, the rats were distributed into five groups; 1-5 of four (4) rats per group. The rats in group 1 were administered with tap water to serve as control while those in groups 4-5 were respectively treated with 200 mg/kg, 400 mg/kg, 600 mg/kg and 800mg/kg body weight of the extract. Treatments lasted for twenty-one consecutive days.

Twenty four (24) hours after the last day of treatment, the animals were sacrificed after exposing them to chloroform vapor. The blood samples were collected by cardiac puncture and transferred into different sample bottles for measurement of parameters.

The haemoglobin evaluation was done by the method described by Okunade, (2012).

0.02ml (20ul) of blood was added to 5ml of Drabkins solution in a test tube. The solution was properly mixed and allowed to stand for 10mins, after which the absorbance was read colorimetrically at 540nM (green filter) with Drabkins solution as blank. The absorbance of the standard was read the same way, and the results were read off using a table prepared from a calibration graph.

The Packed Cell Volume (PCV) was determined according the method described by Singh (2019). The capillary tubes used for PCV was filled with blood**,** one end of the capillary tube was sealed with plastacine. The sealed end was placed to the center of the haematocrit centrifuge and was spun at 12000g for 5mins**.** The spun tube was placed on a designed scale and the parked cell volume was read out as percentage.

Evaluation of blood sugar was done by the glucose oxidase method (Tietz, 1995)**,** while serum urea was measured by the kinetic urease method (Armbruster, 1993).

The histological investigation was according to the method described by Moreira *et al*., (2017).

The pancreas was excised and observed grossly. Pancreas tissues was fixed in buffered formalin and then embedded in paraffin. Sections (4 µm) from the paraffin blocks was prepared and stained with hematoxylin and eosin (H&E) procedure. Histological examination of pancreas was performed by evaluating the degree of insulitis on Langerhans islets at 400x magnification, consistent across 10 fields of view.

STATISTICAL ANALYSIS

Experimental data was expressed as Mean ± Standard Deviation (SD) for quadruplicate determinations. The analysis of variance (ANOVA) for a completely randomized design was used and the values were considered significant at $P<0.05$.

RESULTS

Table 1: Effect of aqueous leaf extract of *Spinacia oleracea* on Serum Urea, blood sugar, Hb and PCV levels of albino rats.

Groups	Urea (mg/dl)	Blood Sugar (mg/dl)	Hb (g/dL)	PCV (%)
Group 1	7.83 ± 0.21^{a}	112.25 ± 9.57^{b}	12.57 ± 0.09^{d}	43.12 ± 0.15^{f}
Group 2	7.71 ± 0.34^{a}	109.00 ± 1.83^{b}	12.65 ± 0.12^{d}	43.17 ± 0.12^{f}
Group 3	7.58 ± 0.33^{a}	107.75 ± 5.80^{b}	12.82 ± 0.05^{d}	43.37 ± 0.27^{f}
Group 4	7.43 ± 0.29^{a}	105.75 ± 2.63^{b}	12.95 ± 0.19^{d}	43.95 ± 0.32^{g}
Group 5	7.25 ± 0.39^{a}	95.25 ± 7.09^{c}	13.30 ± 0.25^{e}	44.01 ± 0.11^{g}

Values represent Mean ± SD (n=4)

a-g: Values in the same column with different superscripts are significantly different ($p<0.05$)

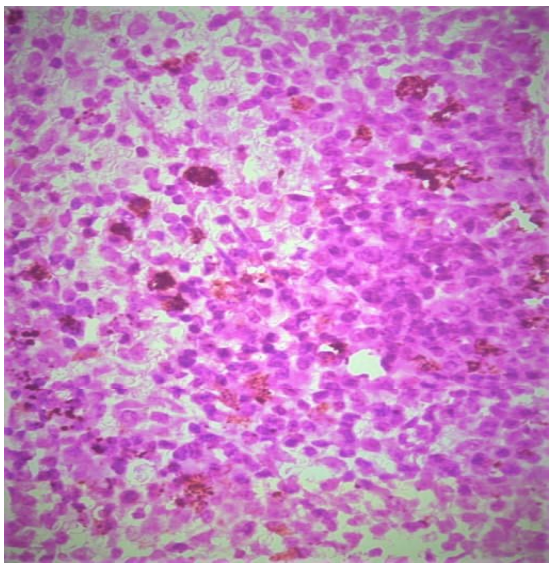

Plate 1: Section of Pancreas of rat kept on feed and tap water only (Control group), Showing well preserved pancreatic accini typical of a healthy pancreas (H&Ex400)

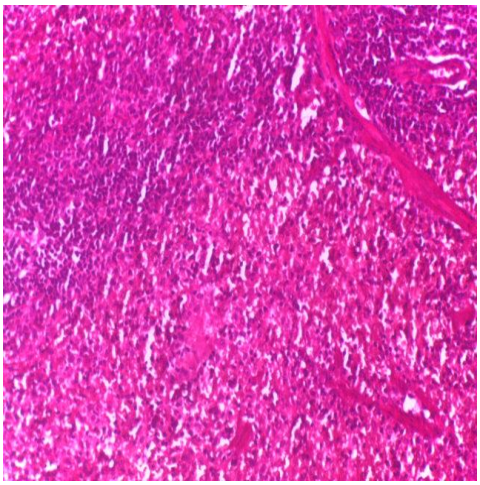

Plate 2: Section of Pancreas of rat treated with *Spinacia oleracea* at the dose of 200mg/kg b.w showing essentially preserved pancreatic accini. (H&Ex400)

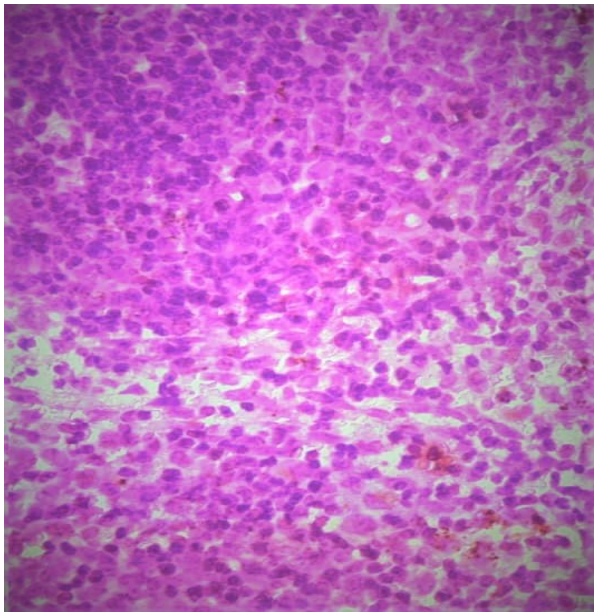

Plate 3: Section of Pancreas of rat treated with *Spinacia oleracea* at the dose of 400mg/kg b.w showing essentially preserved pancreatic accini. (H&Ex400)

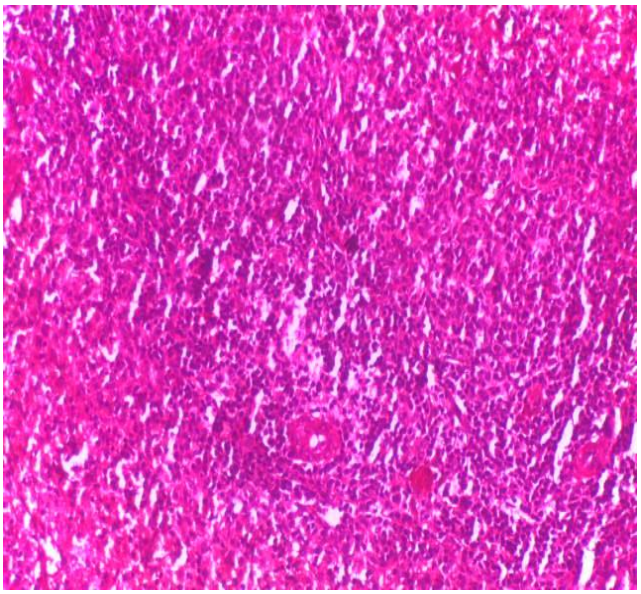

Plate 4: Section of Pancreas of rat treated with *Spinacia oleracea* at the dose of 600mg/kg b.w showing essentially preserved pancreatic accini. (H&Ex400)

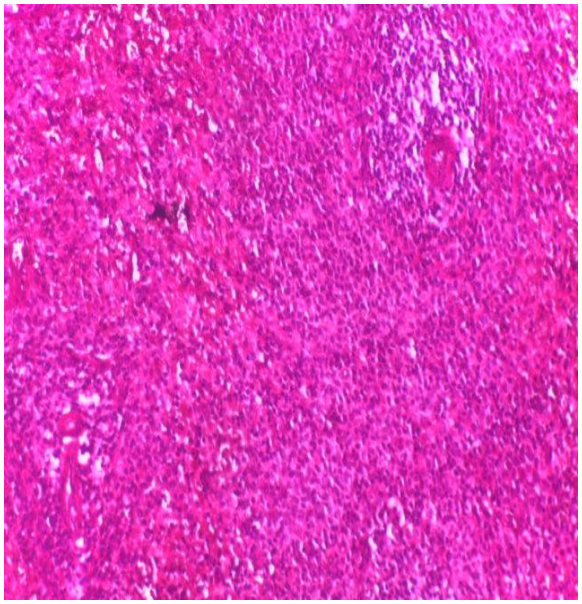

Plate 5: Section of Pancreas of rat treated with *Spinacia oleracea* at the dose of 800mg/kg b.w showing essentially preserved pancreatic accini. (H&Ex400)

DISCUSSION AND CONCLUSION

The results indicate a dose-dependent increase in both hemoglobin (Hb) concentration and packed cell volume (PCV) following the administration of aqueous leaf extract of ***Spinacia oleracea*** at different doses. These findings are significant in evaluating the hematopoietic potential and safety profile of the plant.

Hemoglobin levels increased progressively across the treatment groups, with rats in group 5 having the highest value. The ability of ***Spinacia oleracea*** to boost hemoglobin levels is likely due to its rich content of iron folate and vitamin c which have been documented to improve erythropoiesis (Gaur *et al*., 2017). Vitamin C enhances the absorption of iron which is a key component of haemoglobin. Folate supports DNA synthesis during erythropoiesis (Akhtar *et al*., 2018).The observed increase in hemoglobin may also be attributed to the antioxidant properties of ***Spinacia oleracea***, which protect red blood cells from oxidative damage. This aligns with findings by Alok *et al.,* (2020), who reported that dietary antioxidants promote erythropoiesis and maintain the structural integrity of the erythrocytes. The dose-dependent effect observed suggests that higher doses provide a more pronounced benefit, possibly due to the cumulative bioavailability of active compounds like carotenoids and flavonoids. Packed cell volume reflects the percentage of red blood cells in blood and is a marker of oxygen-carrying capacity. The significant increase in PCV with increasing doses of ***Spinacia oleracea*** corroborates the findings with haemoglobin, further supporting its

erythropoietic potential. As with hemoglobin, the enhancement of PCV may stem from the bioactive compounds in ***Spinacia oleracea*** that prevent oxidative stress and promote erythopoiesis. (Shukla *et al*., 2018).Interestingly, the differences among the groups suggest that doses of 600 mg/kg and 800 mg/kg are particularly effective in augmenting PCV. This is attributable to the synergistic action of micronutrients and phytochemicals in ***Spinacia oleracea***, as reported by Pandey et al. (2019), who emphasized that dietary antioxidants improve hematological parameters. Urea is a waste product created when dietary protein is broken down in the body. Abnormal levels of urea in the blood may indicate hepatic or renal disease as well as dehydration and other conditions. Treatment of the rats with the extracts of ***Spinacia oleracea*** resulted to slight reductions in serum urea levels of the rats which indicates a positive /protective effect on the renal cells. The same treatment also yielded dose- dependent reductions in blood sugar, with the reduction becoming significant ($p<0.05$) when the highest dose of the extract was introduced. This observation suggests that the extract exerts a hypoglycemic effect on the animals. Histological investigation of the pancreas across the treatment groups showed no adverse effects owing to the administration of the extract. The pancreas histology remained well-preserved in all groups, with no evidence of inflammation, necrosis, or structural damage. This indicates that the plant extract is safe for (and protects) the pancreas, even at higher doses, an observation which collaborates with the observed effect on blood sugar.

The pancreas in the control group exhibited typical acinar structures and healthy tissue architecture, reflecting the baseline state of a normal pancreas. This finding serves as a reference for evaluating the effects of the extract .across all treatment groups, the pancreatic acini remained essentially preserved, indicating that ***Spinacia oleracea*** does not induce histological alterations. This is consistent with already existing knowledge that plant-based diets rich in antioxidants could protect tissues from oxidative and inflammatory damage. The preservation of pancreatic tissue integrity, even at the highest dose of 800 mg/kg, suggests that ***Spinacia oleracea*** has no toxic effects on the pancreas. This aligns with studies by Kumar et al. (2021), which highlighted the potency of plant- based bioactive compounds at protecting the liver and pancreas.

The histological preservation of the pancreas may be attributed to the high content of antioxidants, such as beta-carotene, lutein, and zeaxanthin, in ***Spinacia oleracea*** (Zhang *et al*., 2019). These compounds act against free radicals and prevent oxidative stress and as

such, protect the pancreas. Additionally, the anti-inflammatory effects of ***Spinacia oleracea*** may inhibit inflammatory pathways that could otherwise compromise pancreatic integrity (Gupta *et al*., 2018).The lack of histological changes at higher doses also suggests that the plant extract does not exert any pro-inflammatory or cytotoxic effects on the pancreas, making it a safe candidate for therapeutic use. The beneficial effects of ***Spinacia oleracea*** on hemoglobin, PCV, and pancreatic cellular integrity can be attributed to its phytochemical composition and multifaceted biological activities.

While this study focused on healthy rats, the preserved pancreatic histology suggests that ***Spinacia oleracea*** could have implications for diabetes management. The pancreas plays a central role in glucose regulation through insulin secretion, and preserving its integrity is critical for preventing or managing diabetes. The study demonstrates that the herbal extract significantly improves hematological parameters (Hb and PCV) in a dose-dependent manner and preserves pancreatic histology across all treatment groups up to the highest dose studied. These effects are likely mediated by the plant's rich antioxidant, anti-inflammatory, and nutritional properties. The findings reveals the potential of ***Spinacia oleracea*** as a safe and effective natural supplement for enhancing hematological health and protecting pancreatic integrity.

REFERENCES

1. Akhtar, S., Ahmed, A., Randhawa, M. A., et al. Nutritional and therapeutic perspectives of spinach **(**Spinacia oleracea**).** *Journal of Food Science and Technology*, 2018; 55(9): 3105-3115.
2. Alok, S., Jain, S. K., and Verma, A. Plant-derived antioxidants: Therapeutic avenues for protecting pancreatic and hematological health. *Antioxidants*, 2020; 9(8): 720.
3. Armbruster, D.A. "Kinetic urease method for urea nitrogen: an-NHS approved method". Anals of Clinical Biochemistry, 1993; 30(5): 529-535. Doi: 10.1177/000456329303000513
4. Bosi, C.F., Rosa, D.W., Grougnet, R., Lemonakis, N., Halabalaki, M., Skaltsounis, A.L. and Biavatti, M.W. Pyrrolizidine alkaloids in medicinal tea of Spinacia oleracea. Brazilian Journal of Pharmacognosy, 2013; 23(3): 425-432.
5. Dash, G.K. and Murthy, P.N. Wound healing effects of Spinacia oleracea Linn. Int J Pharm Bio Sci., 2011; 2(2): 369-383.

6. Gupta, R., Gupta, N., & Gupta, A. K. Antioxidant and anti-inflammatory activities of **Spinacia oleracea**. *Plant Foods for Human Nutrition*, 2018; 73(3): 233-240.
7. Jain, S. and Puri, H.S. Ethnomedicinal plants of Jaunsar-Bawar hills, Uttar Pradesh. Ind. J. Ethnopharmacol., 2020; 12: 213-220.
8. Kaur, R., Singh, B. and Kaur, S. Pharmacognostic stud-ies on leaves of Spinacia oleracea Linn. Journal of Pharmacognosy and Phytochemistry, 2018; 7(3): 3181-3185.
9. Khare, C.P. Indian Medicinal Plants: An Illustrated Dictionary. Allahabad (India): Lalit Mohan Basu, 2017; 2(7): 34-66.
10. Kohler, J.C. and Baghdadi-Sabeti, G. (2011). The World Medicines Situation, 3rd Ed, World Health Organization.
11. Kumar, A., Singh, M., & Shrivastava, S. Evaluation of hepatoprotective and pancreatic-protective properties of leafy greens. *Nutrition and Metabolism*, 2021; 18(1): 56.
12. Moreira, M.D., Picanço, M.C., Barbosa, L.C.A., Guedes, R.N.C., Barros, E.C. and Campos, M.R. Compounds from Spinacia oleracea: Isolation, structure elucidation and insecticidal activity. Pest Manag Sci., 2017; 63(6): 615621.
13. National Institute of Health Guide for the Care and Use of Laboratory Animals. DHEW Publication, No. (NIH), 1978; 78-23.
14. Okunade, A.L. Spinacia oleracea L. (Asteraceae). Fitoterapia, 2012; 73: 1-16.
15. Pandey, K. B., & Rizvi, S. I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, 2019; 3191861.
16. Singh, H. Ethnobiological treatment of Piles by Bhoxas of Uttar Pradesh. Ancient Sci. Lif., 2019; 8: 16-77.
17. Shukla, S., Chatterjee, S., & Singh, S. P. Phytochemical and therapeutic evaluation of **Spinacia oleracea**. *International Journal of Food Sciences and Nutrition*, 2018; 69(5): 561-570.
18. Tietz, N.W. (1995). Clinical Guide to laboratory Tests (3rd ed.). W.B. Saunders cCompany. ISBN 978-0721648744
19. Woldeyes, S., Adane, L., Tariku, Y., Muleta, D. and Begashaw, T. Evaluation of antibacterial activities of compounds isolated from Sida rhombifolia Linn. (Malvaceae). Nat Prod Chem Res., 2012; 1(101): 2-13.
20. Zang, Y. Zhang, L./ Yang, B., Hu, X., Li, R. and Chu, X. Antioxidant activity of extracts from Spinacia oleracea (spinach) and its active compounds. Journal of Food Science and Technology, 2019; 56(2): 831-839. Doi: 10.1007/s13394-018-2944-6