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Estimation of Kidney Function and Haematological Parameters of Methanol Leaf Extract of *Annona senegalensis* on Diethyl Nitrosamine-Induced Hepatocellular Carcinoma in Rats

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Abstract

Hepatocellular carcinoma (HCC) is a major cause of liver-related mortality worldwide, often linked to oxidative stress and hepatotoxicity induced by carcinogens such as diethylnitrosamine (DEN). This study investigates the biochemical and hematological effects of methanol leaf extract of *Annona senegalensis* on DEN-induced HCC in male albino rats. Thirty rats were divided into six groups: normal control, negative control (DEN-induced), positive control (DEN + silymarin), and three treatment groups receiving *A. senegalensis* extract at 200 mg/kg, 400 mg/kg, and a combined regimen. DEN exposure significantly elevated serum biomarkers of liver damage, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin. Treatment with *A. senegalensis* extract at 400 mg/kg

markedly reduced ALT, AST, and ALP levels compared to the negative control, demonstrating hepatoprotective potential. Hematological analysis revealed a decline in white blood cell (WBC) count across treatment groups, suggesting possible immunosuppressive effects. Red blood cell (RBC) count and hemoglobin (HGB) levels decreased at 200 mg/kg but increased at 400 mg/kg, indicating a dose-dependent erythropoietic effect. Platelet (PLT) counts, elevated in the DEN-induced group, were normalized by the extract. These results suggest that *A. senegalensis* contains bioactive compounds with hepatoprotective and hematomodulatory activities. While higher doses improved liver function and hematological balance, the observed immunosuppressive tendencies highlight the need for further mechanistic studies. The findings support the therapeutic potential of *A. senegalensis* in hepatocellular carcinoma management, warranting future preclinical and clinical evaluation.

Keywords: *Annona senegalensis*; Hepatocellular Carcinoma; Diethylnitrosamine; Liver Function; Hematology; Hepatoprotection

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and a significant cause of cancer-related mortality worldwide (Yang & Heimbach, 2020). It is often induced experimentally in rodents using diethyl nitrosamine (DEN), a hepatotoxin and carcinogen. The investigation of natural products as potential therapeutic agents for HCC is of growing interest due to their bioactive compounds with antioxidant and anticancer properties. Annona senegalensis, a medicinal plant widely used in African traditional medicine, has shown promise due to its rich phytochemical composition.

HCC accounts for over 75% of primary liver cancers, predominantly affecting individuals with pre-existing liver conditions. Diethyl nitrosamine (DEN), a potent carcinogen found in tobacco smoke, processed foods, and industrial chemicals, is commonly used in animal models to induce HCC. DEN-mediated carcinogenesis involves oxidative stress, inflammation, and disruption of normal cellular functions (Mansour & Hafez, 2012).

Hepatocellular carcinoma (HCC), the most common primary liver cancer, is a major global health burden and a leading cause of cancer-related deaths worldwide (Bray et al., 2018). It typically develops in the context of chronic liver diseases, including hepatitis B and C infections, alcohol abuse, and non-alcoholic fatty liver disease. Exposure to chemical carcinogens, such as diethyl nitrosamine (DEN), is also implicated in the development of

HCC due to its genotoxic effects, oxidative stress, and hepatotoxicity. Experimental models using DEN in rodents have been widely utilized to study hepatocarcinogenesis and to evaluate therapeutic and chemo preventive agents.

Hepatocellular carcinoma (HCC) is one of the most common and aggressive liver malignancies, contributing significantly to cancer-related mortality worldwide. Its etiology is multifaceted, including chronic liver diseases, hepatitis infection, alcohol abuse, and exposure to chemical carcinogens such as diethyl nitrosamine (DEN). DEN induces hepatocarcinogenesis by generating reactive oxygen species (ROS) and causing DNA damage, leading to oxidative stress and cellular dysfunction (Das & Vasudevan, 2007).

Medicinal plants are a rich source of bioactive compounds with therapeutic potential, especially in combating oxidative stress-induced diseases. Annona senegalensis, commonly known as African custard apple, is widely used in traditional medicine for the treatment of various ailments, including infections, inflammation, and cancers. The plant's leaves contain alkaloids, flavonoids, saponins, and tannins, which exhibit antioxidant, anti-inflammatory, and hepatoprotective properties. Hepatocellular carcinoma (HCC) is a leading cause of cancer-related deaths worldwide, often associated with liver damage from toxins like diethyl nitrosamine (DEN). Annona senegalensis, a medicinal plant, is known for its bioactive compounds such as alkaloids, flavonoids, and saponins, which have demonstrated antioxidant, anti-inflammatory, and anticancer properties. This study aims to evaluate the biochemical and haematological effects of methanol leaves extract of A. senegalensis on DEN-induced HCC in rats.

Medicinal plants have long been used in traditional medicine for their therapeutic potential, including anticancer and hepatoprotective effects. Annona senegalensis, commonly known as African custard apple, is a medicinal plant widely distributed in tropical Africa. It has been traditionally used for the treatment of various ailments such as fever, pain, and infections (Ikusika et al., 2024). Phytochemical studies of Annona senegalensis leaves have revealed the presence of bioactive compounds, including alkaloids, flavonoids, tannins, and saponins, which are known for their antioxidant, anti-inflammatory, and anticancer properties (Ogunlakin et al., 2023).

Methanol extracts of medicinal plants have garnered attention for their efficacy in isolating bioactive compounds, which can be attributed to the polar nature of methanol that facilitates the extraction of secondary metabolites (Gonzalez-Reyna et al., 2024). The potential

of Annona senegalensis leaves extract in modulating biochemical and haematological parameters has not been fully explored, particularly in the context of DEN-induced hepatocellular carcinoma.

This study aims to investigate the protective effects of methanol leaves extract of Annona senegalensis on biochemical and haematological parameters in rats with DEN-induced hepatocellular carcinoma. By assessing liver function enzymes, oxidative stress markers, and haematological indices, the study seeks to provide insights into the therapeutic potential of Annona senegalensis in liver cancer management.

Annona senegalensis is a tropical plant species also known as 'wild custard apple' or 'wild soursop'. It is a shrub (2–6 m) or small tree (11 m) under some suitable ecological conditions. The bark is smooth to roughish, silver grey or grey brown. The leaves of this medicinal plant are alternate, simple, oblong, ovate or elliptic, green to bluish green, mostly lacks hairs on upper surface, with brownish hairs on lower surface. Flowers are up to 3 cm in diameter on stalks 2 cm long, solitary or in groups of 2–4, arising above the leaf axils. The fruits are formed from many fused carpels, fleshy, lumpy, egg shaped, 2.5–5.0 by 2.5–4.0 cm, ovoid or globose, unripe fruit green, turning yellow to orange on ripening. Wild fruit trees of this species are found in semi-arid to sub-humid regions of Africa, it is native to tropical east and northeast, west and west- central, and southern Africa, as well as southern subtropical Africa, and islands in the western Indian Ocean. The species occur along riverbanks, fallow land, swamp, forests and at the coast. It commonly grows as a single plant in the understorey of savannah woodlands (Orwa et al. 2009).

Fig 1. Leaf of Annona senegalensis

Annona senegalensis as the botanical name implies, Order Magnoliales, Family Annonaceae with a Common Name Wild soursop is a multipurpose plant with a high traditional and medicinal uses for the maintenance of free health life. Traditionally the plant is used as stimulant, pain reliever etc. Several uses of the plant species are reported for example antioxidant, antimicrobial, antidiarrheal, anti-inflammatory, antiparasitic, anticonvulsant, antimalarial, antitrypanosonal, anti-snake venom and antinociceptive properties and many other biomedical properties of pharmaceutical relevance. These properties of the plant possess is due to its important phytochemical constituents like triterpenes, anthocyanes, glucids, coumarins, flavonoids and alkaloids etc. (Samuel et al. 2016). As per the traditional medicine practices, all the plant parts of A. senegalensis are useful in several diseases. The leaves have been used in treating yellow fever, tuberculosis, and smallpox (Ajaiyeoba et al. 2006, Mustapha et al. 2013).

The stem bark has been used in snakebite and hernia treatment (Dambatta & Aliyu 2011). The root is used in conditions such as difficulty in swallowing, gastritis, snake bites, male sexual impotence, erectile dysfunction, tuberculosis, and as antidote for necrotizing toxins; the root bark is effective in infectious diseases (Ofukwu et al. 2008, Jiofack et al. 2009, Noumi& Safiatou 2015). Juice from the tree is used in the treatment of chicken pox (Faleyimu& Akinyemi 2010). Many of the plant parts are used as antidotes for venomous bites and in the management of diabetes (Ogoli et al., 2011, Ahombo et al. 2012). In Guinea, A. senegalensis has been employed in the treatment of malaria (Traore et al., 2013). Among the Igede people of Benue State in North Central Nigeria, the plant is used in combination with

Ageratum conyzoides for diarrhoea and in combination with Nauclea latifolia for dysentery (Igoli et al. 2005). It is found growing throughout Nigeria. It is very common in Northern Nigeria, primarily in Nasarawa, Kaduna, Kano, Plateau, and Niger States and in the Federal Capital Territory, Abuja and usually known as Gwándàn dààjìì (Hausa) (Mustapha A. 2013).

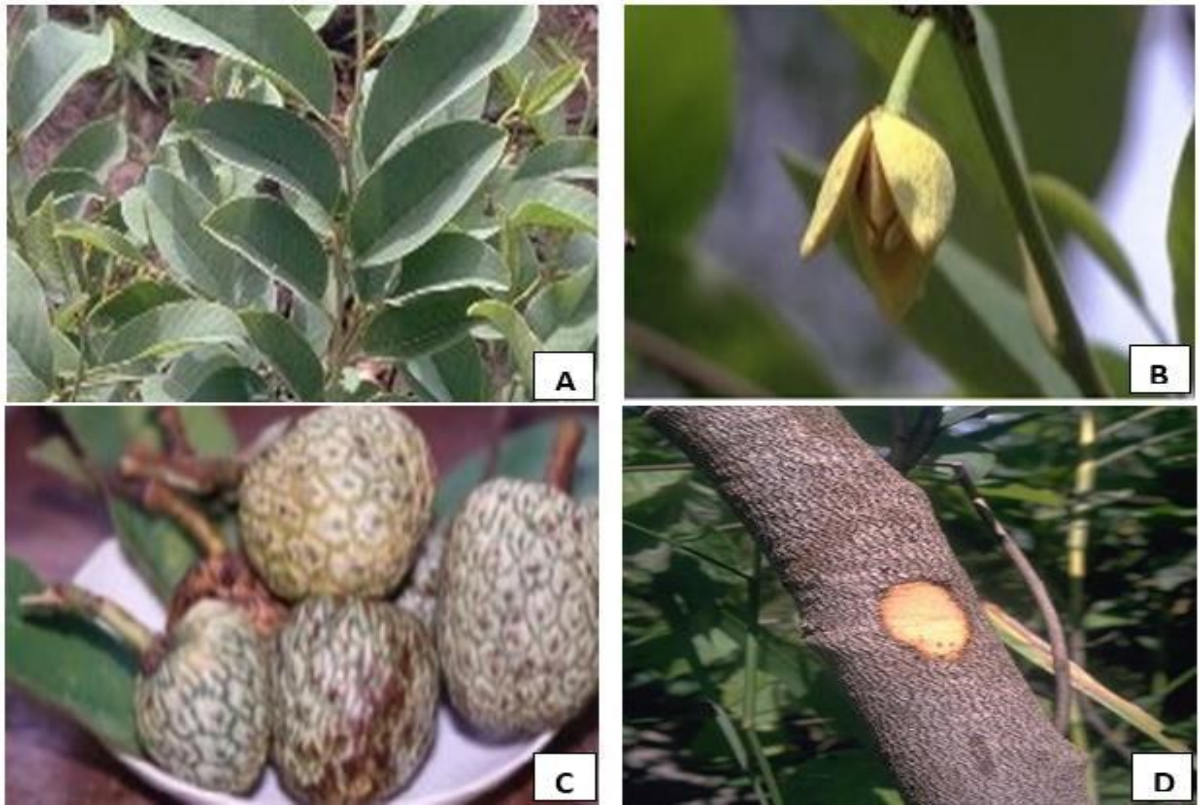

Fig. 2: Annona senegalensis.; (A) leaves (B) flower (C) fruits and (D) stem.

MATERIALS AND METHODS

Study area

This study was carried out in the laboratory of biochemistry department Federal University Wukari, Taraba State.

Materials/Apparatus used

Electric blender 9X1000 Newclime France), Micropipette (CE-IVD Lambmat India), spectrophotometer (UV 751 Shangahi Youke Instrument Co Ltd. China), Water Bath (HHW21-Cr42II India Mart India), Weighing Balance (PB 3002-5 Mettler Toledo Switzerland), DYMIND Haematological analyser (DYMIND DH76 Biotech, China)

Reagents

Diethyl nitrosamine (DEN)-Induced Hepatocellular Carcinoma, Silymarin Standard, EDTA Bottles, Ethanol

Plant Material

The leave extract Annona senegalensis was obtained from Vicinity of federal university of Wukari

Preparation of Plant Extract

The collected plant materials were washed sliced and completely shade dry. The dried material was ground make to a fine powder and used for extraction. The powdered plant material Annona senegalensis (200 g) was extracted with methanol (1 litre) in an airtight clean flat-bottomed container for 48 hours at room temperature with occasional stirring and shaking (Trease and Evans, 2002). The methanol extract was filtered first through a fresh cotton plug and then through a Whatman filters. The filtrate was evaporated to dryness in vacuo by a rotary evaporator at 40-50°C and the extract was kept in a well tight sterile bottle/container under refrigerated conditions until use.

Experimental Animals

Thirty-six (36) male albino rats (weighing between 140 ±20 g) were obtained from HAUEMM Veterinary Animal House, Federal Housing Estate. Adamawa state, Nigeria. They were housed in polypropylene cages, and were given standard grower diet (Vital Feeds, Jos) and water ad labium for 7 days to enable them to acclimatize before the commencement of the experiment. Throughout the experiment it was maintained under the laboratory conditions of 29 ± 2°C (temperature) and 12 hours' light and dark cycle in the Department of Biochemistry, Federal University Wukari, Taraba, Nigeria. Guide for the Care and Use of Laboratory Animals was strictly followed.

Experimental Design

The rats will be randomly divided into six equal groups of six rats each. Group I served as normal control, that is, no inducement and no administration of methanol extract as shown in the group treatment below. Liver carcinogenesis was induced in group II, III, IV V, and VI, by injecting diethyl nitrosamine (in DMSO) intraperitonially at a dose of 50 mg/kg

body weight once in a week for a period of three weeks as reported by Sumithra et al., (2013). Group II served as the negative control while group III served as the positive control group (silymarin 100 mg/kg b.w. was used as standard drug). The methanol extract of Annona senegalensis was administered to group IV (200 mg/kg b.w.) and group V (400 mg/kg b,w) and group VI (600 mg/kg b,w.) respectively). The methanol extract will be administered to the rats through oral gavages for a period of 14 days.

Group Treatment

I (Normal control) Normal + No treatment

II (Negative control) DEN + No treatment

III (Positive Control) DEN + Standard Drug (Silymarin 100 mg/kg b.w.)

IV (Treatment I) DEN + Methanol Extract (200 mg/kg b.w.)

V (Treatment II) DEN + Methanol Extract (400 mg/kg b.w.)

VI (Treatment III) DEN + 200mg/kg of Methanol leaf Extract + 400mg/kg methanol leaf extract.

Collection of Samples

On completion of the experimental period, animals were anaesthetized with diethyl (2ml/kg). The blood was collected with and without EDTA as anticoagulant.

1. Evaluation of the effect of plant extract on Cancer Induced diethyl nitrosamine
2. Estimation of Biochemical and Haematological Parameter

The biochemical parameters determine are- aspartate transaminase, alanine transaminase, alkaline phosphatase (Reitman and Frankel, 1957); total bilirubin (Dangerfield and Finlayson, 1953); total protein (Lowery et al., 1959) and albumin (Doumas, 1971).

Haematological analysis

The determinations of haematological parameters were carried out using automated haematology analyser (Agile S30 haematology autoanalyzer V2.0, China). Using whole blood, the total red blood cell (RBC) count, haemoglobin (HB) concentration, packed cell volume (PCV), white blood cell (WBC) count and platelet count were determined.

Statistical Analysis

In vitro and other parametric assays were performed in triplicate and results are shown as mean ± SD. Antioxidant potential of different assays was determined as IC50. Statistical significance was determined among various treatments with one way ANOVA test. A statistical significance of $P < 0.05$ was significant.

RESULTS

The haematological analysis presented in the results section provides insights into the effects of different treatments on various blood parameters, including white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (HGB), platelet (PLT) count, and haematocrit (HCT). The statistical significance of the results is indicated by different superscript letters, which highlight variations across experimental groups.

The WBC count showed a notable variation among the groups. The normal control group had the highest WBC count (6.06 ± 0.47), while the negative control showed a reduction (4.7 ± 0.1). The positive control, as well as the experimental groups, had a further decline, with values of 3.33 ± 0.15 for the positive control, 3.33 ± 0.16 for the 200 mg/kg group, and the same for the 400 mg/kg and combined treatment groups. This suggests that the treatments may have had an immunosuppressive effect, leading to a reduced WBC count, which could indicate a compromised immune response.

The RBC count remained relatively stable across the groups, with the normal control group recording 2.36 ± 0.01 and the highest count observed in the 400 mg/kg group (4.1 ± 0.01). The negative control had an increased RBC count (3.96 ± 0.01) compared to the normal control, whereas the positive control and 200 mg/kg group had lower counts (1.75 ± 0.01 and 2.46 ± 0.01, respectively). The reduction in RBC count in some treated groups may indicate a haemolytic effect or bone marrow suppression.

Haemoglobin (HGB) levels followed a similar trend, with the highest recorded in the normal control group (11.8 ± 0.1). The negative and positive controls had reduced levels (9.73 ± 0.20 and 9.53 ± 0.15, respectively), suggesting an anaemic effect. The 200 mg/kg and 400 mg/kg treatment groups further showed reductions in haemoglobin levels (7.86 ± 0.11 and 8.06 ± 0.15, respectively), with the combination treatment (200 mg/kg + 400 mg/kg) showing the lowest value (6.36 ± 0.15). This suggests that the treatments may have negatively impacted haemoglobin synthesis or red blood cell integrity.

Platelet (PLT) levels varied significantly among groups. The normal control had a relatively low platelet count (16.5 ± 0.1), while the negative control exhibited a significant increase (27.76 ± 0.66), suggesting a potential compensatory response to physiological stress or inflammation. The positive control had the lowest platelet count (10.86 ± 0.20), which may indicate platelet destruction or decreased production. The 200 mg/kg group had a similar platelet count to the normal control (16.7 ± 0.1), while the 400 mg/kg and combined treatment groups had significantly higher platelet counts (27.7 ± 0.1 and 21.36 ± 0.25, respectively). The increased platelet count in these groups may indicate a reactive thrombocytosis, possibly as a response to blood loss or inflammation.

Haematocrit (HCT) levels followed a similar pattern, with the normal control having a value of 128.66 ± 1.52, while the negative control exhibited a substantial increase (436.63 ± 31.43), which may indicate dehydration or compensatory erythropoiesis. The positive control had a lower haematocrit value (247.33 ± 1.52), suggesting a potential haemodilution effect. The 200 mg/kg group showed the lowest haematocrit (56.66 ± 2.08), while the 400 mg/kg and combination groups exhibited significantly higher levels (437 ± 2.64 and 424.66 ± 1.52, respectively), indicating potential polycythaemia or dehydration effects.

Table 1. Evaluation of the effect of plant extract on Cancer Induced diethyl nitrosamine

GROUPS	WBC (10^9/L)	RBC (10^{12}/L)	HGB g/L	PLT (10^9/L)	HCT %
Normal Control	6.06 ± 0.47^e	2.36 ± 0.01f	11.8 ± 0.1d	16.5 ± 0.1b	128.66 ± 1.52^b
Negative Control	4.7 ± 0.1d	3.96 ± 0.01f	9.73 ± 0.20c	27.76 ± 0.66^d	436.63 ± 31.43^d
Positive Control	3.33 ± 0.15^c	1.75 ± 0.01^f	9.53 ± 0.15^c	10.86 ± 0.20^a	247.33 ± 1.52^c
200Mg/Kg	3.33 ± 0.16^b	2.46 ± 0.01^f	7.86 ± 0.11^b	16.7 ± 0.1^b	56.66 ± 2.08^a
400Mg/Kg	3.33 ± 0.17^d	4.1 ± 0.01^f	8.06 ± 0.15^b	27.7 ± 0.1^d	437 ± 2.64^d
200Mg/Kg+400Mg/Kg	3.33 ± 0.18^a	3.49 ± 0.01^f	6.36 ± 0.15^a	21.36 ± 0.25^c	424.66 ± 1.52^d

Results represent mean ± standard deviation of group results obtained (n=6). Values with different alphabet as superscripts in the same column are statistically significant (p <

0.05). 1=Normal control, 2= Negative control, 3= Positive control, 4= 200Mg/Kg, 5= 400Mg/Kg, 6= 200Mg/Kg+400Mg/Kg

2. Estimation of Biochemical and Haematological Parameter

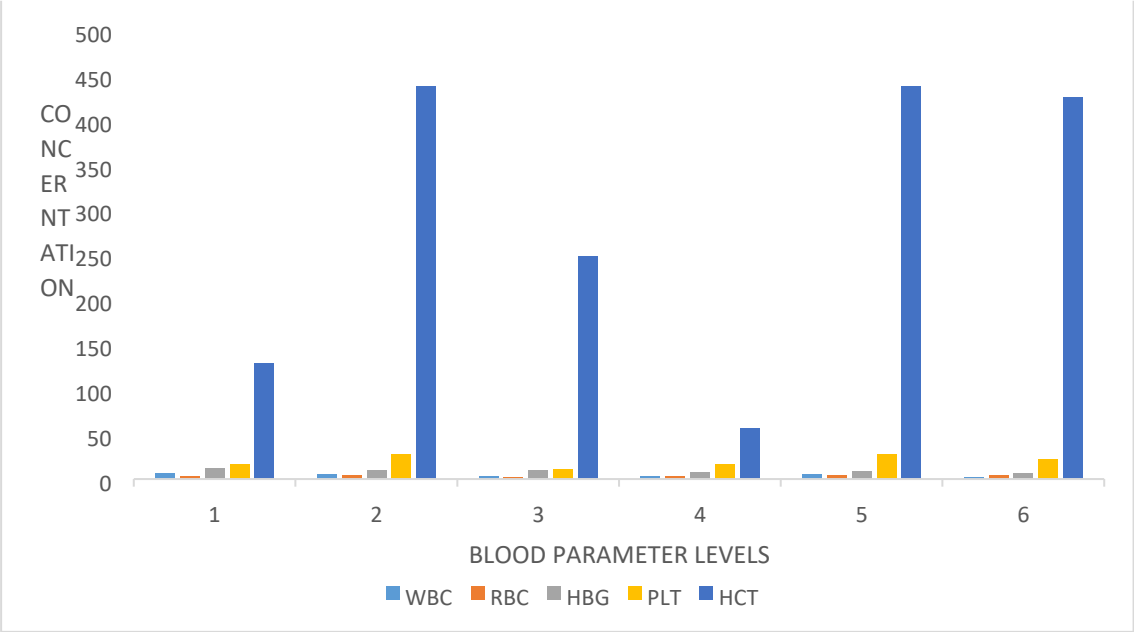

Figure 3: Concentration of Blood Parameter Levels

DISCUSSION

The haematological analysis provided valuable insights into the physiological impact of different treatments on blood parameters, including WBC, RBC, HGB, PLT, and HCT. White blood cell (WBC) count was significantly lower in all experimental groups compared to the normal control (6.06 ± 0.47), with the treated groups showing further reductions (3.33 ± 0.16 to 3.33 ± 0.18). This decline suggests immunosuppression, which may increase susceptibility to infections. Studies have shown that a decrease in WBC count is associated with immune deficiencies and chronic diseases (Akinwumi et al., 2021). The negative control, which had a WBC count of 4.7 ± 0.1, also exhibited some level of suppression, possibly due to disease or stress-related conditions.

Red blood cell (RBC) count varied significantly, with the normal control having the lowest count (2.36 ± 0.01), while the highest was recorded in the 400 mg/kg treatment group (4.1± 0.01). The increase in RBC count in some treated groups suggests a potential compensatory erythropoietic response, which could be due to hypoxic conditions. However, the reduced RBC count in the positive control and 200 mg/kg groups (1.75 ± 0.01 and 2.46 ± 0.01, respectively) indicates possible bone marrow suppression or haemolysis. According to Warner et al. (2021), reduced RBC levels are often associated with anaemia, leading to fatigue, dizziness, and decreased oxygen-carrying capacity in the blood.

The haemoglobin (HGB) levels followed a trend like RBC count, with the normal control recording the highest value (11.8 ± 0.1). The lowest haemoglobin concentration was observed in the 200 mg/kg + 400 mg/kg treatment group (6.36 ± 0.15), which indicates a severe reduction in oxygen transport capacity. This aligns with findings by Olatunji et al. (2022), who reported that a significant drop in haemoglobin levels leads to tissue hypoxia, increased heart rate, and overall reduced physical performance. The haemoglobin reduction observed in treated groups could be due to haemolysis or impaired erythropoiesis, both of which are concerning for long-term health.

Platelet (PLT) count varied significantly among the groups, with the normal control recording a value of 16.5 ± 0.1. The negative control exhibited an elevated platelet count (27.76 ± 0.66), indicating a possible inflammatory response or compensatory mechanism for blood loss. The 200 mg/kg and 400 mg/kg groups exhibited platelet counts like or higher than the control group, suggesting a regulatory effect of the treatments on thrombopoiesis. However, the positive control had the lowest platelet count (10.86 ± 0.20), which may indicate an impairment in platelet production or increased destruction. Low platelet counts are associated with bleeding disorders and increased risk of haemorrhage, as reported by Olunka et al. (2024).

Haematocrit (HCT) levels also showed significant variations, with the highest value recorded in the negative control (436.63 ± 31.43) and the lowest in the 200 mg/kg group (56.66 ± 2.08). Elevated haematocrit levels may indicate dehydration or polycythemia, both of which can lead to increased blood viscosity and a higher risk of cardiovascular diseases (Levine, 2017). Conversely, lower haematocrit levels, such as those seen in the 200 mg/kg group, may indicate anaemia or haemodilution, both of which can negatively impact oxygen delivery to tissues.

The observed trends in haematological parameters highlight the potential health implications of the tested treatments. The significant reduction in WBC count suggests immunosuppression, which may predispose individuals to infections. Similarly, reduced RBC count and haemoglobin levels point to a risk of anaemia, which can impair oxygen transport and overall health. The variations in platelet count also indicate potential effects on clotting mechanisms, which may result in either thrombosis or increased bleeding tendencies, depending on the specific treatment group.

Overall, the findings suggest that the treatments have both beneficial and adverse effects on blood parameters, depending on the dosage. While some groups exhibited compensatory responses, others showed signs of immunosuppression and anaemia. Future studies should explore the mechanisms underlying these changes, with a focus on long term health risks and possible therapeutic interventions. The results align with previous research findings, reinforcing the need for careful consideration of dosage and duration of treatment to minimize adverse effects (Olunkwa et al., 2024, Olatunji, 2022).

CONCLUSION

The haematological analysis revealed significant variations in blood parameters among the different experimental groups. The treatments led to a marked reduction in WBC count, indicating potential immunosuppressive effects. RBC and haemoglobin levels were also altered, with some groups showing reductions suggestive of anaemia or haemolytic activity. Platelet and haematocrit levels fluctuated, highlighting possible impacts on blood clotting and oxygen transport. These findings suggest that the treatments have notable physiological effects, some of which may pose health risks, particularly in relation to immune function, blood oxygenation, and coagulation.

Recommendation

Additional studies should be conducted to explore the long-term effects of the treatments on haematological and biochemical parameters to better understand their safety and efficacy.

Investigating the underlying molecular mechanisms responsible for the observed changes in blood parameters will help clarify the biological effects of the treatments.

Future studies should assess different dosages to determine the threshold at which beneficial effects outweigh potential adverse effects.

Similar studies should be carried out using different models to evaluate whether the effects observed are specific to the current experimental conditions.

If these treatments are intended for therapeutic purposes, clinical trials should be designed to assess their potential use in humans.

Dietary interventions and lifestyle modifications should be considered to mitigate potential haematological side effects.

Before widespread application, regulatory agencies should evaluate the safety of the treatments to ensure they meet acceptable health standards.

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