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Crude and Nanosynthesized Selected Medicinal Plants Demonstrate Low Acute Toxicity and Hematopoietic Efficacy in Wistar Rats

Muftau Kolawole Oladunmoye [1], MacDonald Idu [2], Isiaka Adekunle Amoo [3], Babayemi Olawale Oladejo [1,*], Ruth Ebunoluwa Bodunrinde [4] and Elizabeth Tomilayo Akinterinwa [1]

[1] *Department of Microbiology, Federal University of Technology Akure, Nigeria.*
[2] *Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.*
[3] *Department of Chemistry, Federal University of Technology Akure, Nigeria.*
[4] *Department of Microbiology, Joseph Sarwuan Tarka University, Makurdi, Nigeria.*

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Abstract

This study evaluated the *in vivo* biosafety profile of extracts from *Hibiscus sabdariffa*, *Justicia carnea*, *Zingiber officinale* and honey alongside their nanosynthesized extracts. Acute (single-dose) and 28-day sub-chronic (100, 200, and 400 mg/kg/day) oral toxicity studies were conducted in Wistar rats following OECD guidelines. Assessments included behavioral observations, body temperature and weight changes, hematological parameters and liver markers including Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) using standard laboratory techniques. Acute toxicity test revealed no mortality or significant adverse behavioral changes up to 400 mg/kg. In the 28-day toxicity study, all formulations were generally well-tolerated. The administration of plants at doses of 100, 200, and 400 mg/kg and NPs does not appear to have a biologically significant effect on the body temperature of albino rats over a 28-day period. Also, the acute toxicity of the plant extracts at 400 mg/kg and the NPs at E9 +NPs1 body weight had no observable deleterious effects on the rat's health, temperature and behavioral features. Hematological analyses indicated significant modulatory effects as extracts administration at 28 days, markedly increasing platelet counts; 1128.50 × 10^9/L at 200 mg/kg and RBC counts 7.30 × 10^{12}/L at 100 mg/kg). This showed that these plants could possess pharmacokinetic potentials which could modulate immune response and serve as potential alternative therapy against infection disease agents.

Keywords: *Hibiscus sabdariffa*; *Justicia carnea*; Liver markers; Nanoparticles; Toxicity; *Zingiber officinale*

1. Introduction

The escalating crisis of antimicrobial resistance (AMR) represents one of the most significant threats to modern healthcare, rendering conventional antibiotics progressively ineffective against common pathogens [1]. The antimicrobial activity of plant extracts has garnered much attention in the biomedical research field due to the growing threat over antibiotic resistance and the need for alternative therapeutic agents. Plants are known to produce various array of secondary metabolites, many of which have antimicrobial properties. Many of these compounds have been shown to exhibit antimicrobial activity against a wide range of pathogens, including bacteria, fungi, and viruses. Several studies have demonstrated that combinations of plant compounds can exhibit synergistic or additive effects, where the antimicrobial activity of the mixture is greater than the sum of the activities of its individual components. Plant-derived compounds often act on several targets within microbial cells, thereby making it difficult for pathogens to develop resistance [1,2]. The failure of empirical antibiotic therapy due to resistance necessitates an urgent paradigm shift toward novel therapeutic strategies. One promising avenue is the exploration of agents that, rather than directly

* Corresponding author: Babayemi Olawale Oladejo

targeting the microbe, bolster the host's own defense mechanisms—a strategy known as immunomodulation [3]. Concurrently, strengthening the host's hematopoietic (blood-forming) system is vital for maintaining physiological resilience and ensuring a robust supply of immune cells during infection.

Medicinal plants remain a vast reservoir of complex phytochemicals with diverse pharmacological activities. *Zingiber officinale* (ginger) is widely recognized for its potent anti-inflammatory and immunomodulatory properties, primarily attributed to compounds like gingerols, which modulate critical signaling pathways such as NF-κB [4]. *Hibiscus sabdariffa* (roselle) has a long history in traditional medicine for treating UTIs, with modern studies corroborating its antimicrobial, antioxidant, and immunomodulatory effects [5]. Similarly, *Justicia carnea* is extensively used in African ethnomedicine, lauded for its remarkable hematopoietic ("blood-boosting") properties. Recent *in vivo* studies have validated this traditional use, demonstrating its capacity to significantly increase red blood cell counts, hemoglobin, and packed cell volume, alongside exhibiting anti-inflammatory activity [6].

Despite this therapeutic potential, the clinical application of crude herbal extracts is often hampered by poor phytochemical stability, low bioavailability, and rapid metabolic degradation [7]. Nanotechnology, specifically the green synthesis of nanoparticles using plant extracts, offers a transformative solution. This method uses the plant's own phytochemicals as reducing and capping agents, creating nanosynthesized formulations that enhance stability, improve delivery, and potentiate biological efficacy [8]. Recent studies confirm that nanoparticles synthesized from *Z. officinale* and *H. sabdariffa* exhibit superior antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* compared to crude extracts [9,10].

A significant gap persists between the *in vitro* potentials of these herbal extracts and the requisite *in vivo* data for therapeutic development. While the immunomodulatory and hematopoietic properties are documented, there is a lack of rigorous, comparative *in vivo* studies evaluating their safety and efficacy, especially for their nanosynthesized counterparts, in the context of an active infection. Furthermore, the toxicity profile of these specific formulations, as mandated by OECD guidelines, has not been thoroughly established. Therefore, the aim of this study was to evaluate and compare the acute and sub-chronic toxicity profiles, as well as the hematopoietic and immunomodulatory efficacy, of crude and nanosynthesized extracts from *H. sabdariffa*, *J. carnea*, and *Z. officinale* in honey.

2. Methods

2.1. Plant Collection and Authentication

The plants used in this study were *H. sabdariffa* L. (calyces), *Z. officinale* L. (rhizomes), and *J. carnea* L. (leaves), identified with voucher numbers UIH-23344, UIH-23356 and UIH-23346 respectively. Honey was obtained from the School of Agriculture and Agricultural Technology (FUTA), Nigeria and proximate analysis was conducted on it [11,12].

2.2. Extraction of Plant Materials using Different Solvents

Ethanol, chloroform and hot water extracts were derived from *H. sabdariffa* calyx, *J. carnea* leaves, and *Z. officinale* rhizomes. The plant materials were air-dried at room temperature for 7–10 days to constant weight, then ground into fine powder using a mechanical blender. The dried, powdered material (100 g) was mixed with the respective solvents at a 1:10 ratio (100 g in 1000 mL of solvent) for each solvent and soaked for 72 hours with intermittent agitation to facilitate extraction. The mixture was then filtrated/sieved using muslin cloth followed by Whatman No. 1 filter paper to remove solid residues [13,14]. The filtrates were concentrated under vacuum using a rotary evaporator (RE-52A, Union Laboratories, England) at 37°C to obtain the crude extracts. The 100% stock concentrates of the extracts were obtained and stored in a refrigerator at 4°C in sterile universal bottles until further use.

2.3. Green Nano-synthesis and Physical Characterization

Silver nanoparticle (AgNP) synthesis involved preparing an aqueous solution (1 mM) of silver nitrate ($AgNO_3$) in 250 mL Erlenmeyer flasks, to which plant extract was added for reduction into Ag^+ ions for each type of plant extract. The composite mixture was kept on a magnetic hot plate or microwave oven for complete bioreduction at a power of 300 W for 4 minutes discontinuously to prevent an increase of pressure. In the meantime, the colour change of the mixture from faint light to yellowish brown to reddish brown to colloidal brown was monitored periodically (time and colour change were recorded along with periodic sampling and scanning by UV-visible spectrophotometry) for a maximum of 30 minutes. The reactions were carried out in dark (to avoid photoactivation of $AgNO_3$) at room temperature. Complete reduction of $AgNO_3$ to Ag^+ ions was confirmed by the change in colour from colourless to colloidal brown. After irradiation, the dilute colloidal solution was cooled to room temperature and kept aside for 24 hrs for complete bioreduction and saturation. Then, the colloidal mixture was sealed and stored properly for further use. The formation

of AgNPs was furthermore confirmed by spectrophotometric analysis [15,16]. To isolate the nanoparticles, the colloidal solution was centrifuged at 4000 rpm for 15 min to remove large aggregates, followed by ultracentrifugation at 25,900 rpm for 30 min to pellet the nanoparticles. The pellets were washed thrice with deionized water to remove unbound phytochemicals and redispersed in sterile water for characterization.

2.4. Animal Handling

Ethical approval for the use and handling of animals was obtained from the Center for Research and Development (CERAD) of the Federal University of Technology Akure (FUTA) Ethical Committee for the use and care of laboratory animals. One hundred and twenty (120) Wistar rats (weighing 18–24 g) were used for this study and were purchased at the breeding colony of the Department of Animal Production and Health, Federal University of Technology Akure. The rats were maintained at 25°C on a 12-hour light/dark cycle with access to food and water for two weeks before the commencement of the experiment. Animal handling was in accordance with the rules and regulations prepared by the Washington Institute for Laboratory Animal Research [17].

2.5. Acute Toxicity Assay

Healthy female Wistar rats were used in this study according to the instructions of the Organization for Economic Cooperation and Development (OECD) for acute oral toxicity tests [18]. All animals were fasted overnight but had free access to water and were weighed before administration of the extracts. The animals were randomly divided into five groups (n = 4 per group) for each extract. Group 1 (Control) received distilled water orally; groups 2, 3 and 4 (Acute toxicity) received 100 mg/kg, 200 mg/kg and 400 mg/kg respectively, while group 5 (nanoparticles) received corresponding plant-based nanosynthesized extract [19,20]. The five groups were also reproduced for the plants used: *H. sabdariffa*, *J. carnea* and *Z. officinale*. The animals were then observed for mortality, signs of acute toxicity and behavioural changes (aggression, unusual vocalization, agitation, sedation and somnolence, convulsions, tremors, ataxia, catatonia, paralysis, fasciculation, prostration and unusual locomotion and asphyxia) for the first 30 mins, 1 hr, 5 hrs and finally periodically up to 48 hrs. All experimental animals were individually observed daily for general behaviour and body weight changes, dangerous symptoms and mortality [21,22].

2.6. Sub-Chronic Toxicity Assay

The experiment was conducted according to the protocols described by OECD Guideline 407 [19,23,24] with minor modifications. This was a 28-day sub-chronic toxicity test and a continuation from the acute testing. Prior to treatment, rats were handled individually and carefully examined for abnormal behaviour and appearance. The plant extracts were dissolved in distilled water, and respective nanoparticles were administered orally once a day for 28 consecutive days. Group 1 (Control) received distilled water orally; groups 2, 3 and 4 (Sub-chronic toxicity) received 100, 200 and 400 mg/mL respectively, while group 5 (Nanoparticles) received corresponding plant-based nanosynthesized extract. The five groups were also reproduced for the plants used: *H. sabdariffa*, *J. carnea* and *Z. officinale*. The animals were observed daily during the experimental period for mortality or morbidity, changes in posture, changes in the fur, skin, eyes, mucous membranes and behaviours. At the end of the first 7 days, the first set of animals, at 14 days another set, and at 21 days a different set were bled to assess internal organs; blood samples were taken by retro-orbital puncture using capillary tubes for hematological and liver biochemical studies [25].

2.7. Hematological and Liver Biochemical Test

Blood samples taken in EDTA tubes were used for hematological treatment using an automated hematology analyzer (ABX Pentra XL 80, France). The differential count of leukocytes was performed with light microscopy after haematological staining (fixation with May Grunewald and staining with Giemsa stain. In each case, 100 cells were counted. Blood samples taken in anticoagulant-free tubes were used for biochemical analysis and were centrifuged at 3000 rpm for 10 min. The sera were separated, stored at -20°C and used for evaluation. Liver biochemical parameters such as; Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) were estimated using URIT 8021A automated analyzer (URIT Medical Electronic Group Co., Ltd.) [26].

2.8. Statistical analysis

All experiments were performed in triplicate, data were expressed as mean ± standard error (SE). Differences were analyzed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) test at $p < 0.05$ using SPSS version 25.

3. Results

3.1. Nanosynthesized Crude Plant Extracts

The green synthesis of AgNPs was initially confirmed by visual color changes in the reaction mixtures which confirm the green synthesis of silver nanoparticles (AgNPs). This color shift is a key indicator that silver ions (Ag+) have been reduced to silver atoms (Ag0), forming the nanoparticles (Table 1).

Table 1 Visual Confirmation of Silver Nanoparticle (AgNP) Synthesis

Plant Extract	Color Before Reaction	Color After Reaction (AgNPs formed)
Justicia carnea	Light Green	Reddish-Brown
Zingiber officinale	Light Yellow	Dark Brown
Hibiscus sabdariffa	Light Purple	Dark Brown

3.2. Behavioural Observations of Oral Acute Toxicity in Rats administered with Nanosynthesized Crude Extracts

The appearance and behavioral observations of the oral administration of *H. sabdariffa* ethanol *J. carnea* hot water, *Z. officinale* hot water extract and their nanoparticles (NPs) in rats is shown in Table 2. There was no observable changes in the body weight, temperature, eye colour and rate of respiration. In addition, there was no lethality and unconsciousness record in any of the experiment groups. However, sedation, slight reduction in food intake and raised fur of rats were observed only immediately after administration which normalizes within 1-2 hours.

3.3. Effects of Administration of *H. sabdariffa* Ethanol Extract on Body Temperature of Albino Rats

Throughout the study, the temperature ranged between 36°C and 39°C across all groups, which is within the normal range for rats and there were no clear dose-dependent trend in temperature changes. Both 200 and 400 mg/kg groups showed significantly higher temperatures than 100 mg/kg and control groups on the last day of the experiment. By day 28, the higher doses (200 and 400 mg/kg) resulted in elevated temperature ranges compared to the lower dose and control as shown in Table 3.

3.4. Effects of Administration of *J. carnea* hot water Extract on Body Temperature of Albino Rats

All groups, including the control, showed some fluctuations in temperature ranges over the 28-day period. These fluctuations appeared to be within a relatively narrow range of 36.5°C to 38.5°C. There was no consistent pattern of temperature increase or decrease over time in any of the treatment groups compared to the control, suggesting that the *J. carnea* hot water extract does not have a pronounced long-term effect on body temperature as shown in Table 4.

3.5. Effects of Administration of *Z. officinale* hot Water Extract on Body Temperature of Albino Rats

There was no clear dose-dependent effect on temperature ranges across the different dosage groups (100 mg/kg, 200 mg/kg and 400 mg/kg) compared to the control. There are no consistent patterns of temperature increase or decrease over time in any of the treatment groups compared to the control. The fluctuations appear random and similar across all groups as shown in Table 5.

3.6. Effects of Administration of Nanoparticles of *H. sabdariffa*, *J. carnea* and *Z. officinale* Extracts on Body Temperature of Albino Rats

There was no significant differences ($P<0.05$) between treatments and control for most days. No consistent pattern of temperature increase or decrease over time for any treatment. Fluctuations appeared random and mostly within the range of control group variations. Treatment group nHSE showed the highest temperature value (38.15°C on Day 7) while nJCH, was generally similar to control, with a slight decrease on day 22 (36.20°C). Group nZOH was closest to the control values throughout the study as shown in Table 6.

Table 2 General Appearance and Behavioural Observations of Oral Acute Toxicity in Rats administered with Plant Extracts and Nanoparticles

Observations	Control	Experimental Groups (HSE)			Experimental Groups (JCH)			Experimental Groups (ZOH)			Experimental Groups (NPs)		
		100 mg/kg	200 mg/kg	400 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	HSE	JCH	ZOH
Body weight	N	C	C	C	C	C	C	C	C	C	C	C	C
Temperature	N	N	N	N	N	N	N	N	N	N	N	N	N
Food/water intake	N	N	N	R	N	N	N	N	N	R	N	R	R
Rate of Respiration	N	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Foot thickness	N	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Change in skin	N	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Eye colour	N	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Raised fur	N	NP	P	P	NP	P	P	NP	P	P	NP	NP	NP
Drowsiness	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Coma	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Death	A	A	A	A	A	A	A	A	A	A	A	A	A

Keys: HSE= *H. sabdariffa* Ethanol Extract, JCH= *J. carnea* Hot Water Extract, ZOH= *Z. officinale* Hot Water Extract, NPs= Nanoparticles, A= Alive, C= Change, NC= Not change, NE= No effect, NP= Not present, R= Reduce, P= Present

Table 3 Effects of Administration of *H. sabdariffa* Ethanol Extract on Body Temperature of Albino Rats

Days	100 mg/kg	200 mg/kg	400 mg/kg	Control
1	38.20±0.35a	37.98±0.83a	38.30±0.79a	36.85±0.05a
4	36.68±0.14a	36.95±0.30a	38.23±0.17b	36.15±0.15a
7	37.48±0.58a	37.60±0.29a	37.30±0.47a	36.35±0.35a
10	37.53±0.29ab	37.00±0.06a	38.23±0.23b	37.10±0.10a
13	37.20±0.42a	37.67±0.27a	37.60±0.60a	37.80±0.10a
16	37.65±1.05a	37.45±0.25a	37.25±0.65a	36.40±0.40a
19	37.60±1.20a	37.50±0.30a	37.15±0.75a	37.05±0.05a
22	37.10±0.10b	36.25±0.25ab	36.00±0.00a	37.25±0.25b
25	37.25±0.25a	37.40±0.10a	37.25±0.25a	36.85±0.05a
28	37.50±0.00b	39.10±0.10c	39.10±0.10c	36.50±0.00a

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different ($p<0.05$) according to Tukey's honestly significant difference

Table 4 Effects of Administration of *J. carnea* hot water Extract on Body Temperature of Albino Rats

Days	100 mg/kg	200 mg/kg	400 mg/kg	Control
1	38.53±0.34^{a}	37.20±0.70^{a}	37.15±0.70^{a}	36.85±0.05^{a}
4	36.58±0.08^{a}	37.53±0.54^{a}	37.33±0.33^{a}	36.15±0.15^{a}
7	36.43±0.30^{a}	37.50±0.11^{b}	37.10±0.17^{bc}	36.35±0.35^{a}
10	37.40±0.12^{a}	36.70±0.70^{a}	37.27±0.23^{a}	37.10±0.10^{a}
13	37.17±0.28^{a}	38.04±0.43^{a}	37.37±0.22^{a}	37.80±0.10^{a}
16	37.80±0.10^{a}	37.45±0.35^{a}	37.35±0.35^{a}	36.40±0.40^{a}
19	37.15±0.05^{a}	37.20±0.60^{a}	37.10±0.00^{a}	37.05±0.05^{a}
22	37.75±0.05^{b}	36.80±0.10^{a}	37.80±0.00^{b}	37.25±0.25^{ab}
25	37.65±0.05^{b}	37.10±0.10^{a}	37.80±0.00^{b}	36.85±0.05^{a}
28	37.05±0.05^{b}	36.80±0.10^{ab}	38.20±0.00^{c}	36.50±0.00^{a}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different ($p<0.05$) according to Tukey's honestly significant difference

Table 5 Effects of Administration of *Z. officinale* hot Water Extract on Body Temperature of Albino Rats

Days	100 mg/kg	200 mg/kg	400 mg/kg	Control
1	37.58±0.55^{a}	37.33±0.74^{a}	36.88±0.05^{a}	36.85±0.05^{a}
4	37.33±0.74^{a}	36.88±0.56^{a}	37.58±0.55^{a}	36.15±0.15^{a}
7	36.88±0.56^{a}	36.88±0.56^{a}	37.03±0.19^{a}	36.35±0.35^{a}
10	37.57±0.07^{a}	37.73±0.38^{a}	37.13±0.43^{a}	37.10±0.10^{a}
13	37.17±0.28^{a}	36.70±0.69^{a}	37.27±0.09^{a}	37.80±0.10^{a}
16	37.80±0.10^{a}	37.65±0.15^{a}	37.15±0.95^{a}	36.40±0.40^{a}
19	37.00±0.10^{a}	37.75±0.35^{a}	37.65±1.45^{a}	37.05±0.05^{a}
22	37.15±0.15^{a}	37.35±0.35^{a}	37.15±0.15^{a}	37.25±0.25^{a}
25	37.20±0.20a	37.35±0.35a	37.40±0.00a	36.85±0.05a
28	36.35±0.35a	37.45±0.45a	36.70±0.00a	36.50±0.00a

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different ($p<0.05$) according to Tukey's honestly significant difference

Table 6 Effects of Administration of Nanoparticles of *H. sabdariffa*, *J. carnea* and *Z. officinale* Extracts on Body Temperature of Albino Rats

Days	nHSE	nJCH	nZOH	Control
1	37.58±0.83^{a}	37.50±0.97^{a}	37.73±0.70^{a}	36.85±0.05^{a}
4	37.18±0.35^{a}	37.08±0.15^{a}	37.50±0.97^{a}	36.15±0.15^{a}
7	38.15±0.60^{a}	37.73±0.70^{a}	37.03±0.47^{a}	36.35±0.35^{a}
10	37.53±0.38^{a}	37.73±0.23^{a}	37.43±0.40^{a}	37.10±0.10^{a}
13	37.37±0.23^{a}	36.78±0.37^{a}	36.87±0.75^{a}	37.80±0.10^{a}
16	37.45±0.05^{a}	37.15±0.05^{a}	36.75±0.05^{a}	36.40±0.40^{a}

19	37.50±0.60[a]	37.05±0.15[a]	36.45±0.65[a]	37.05±0.05[a]
22	37.05±0.50[ab]	36.20±0.20[a]	37.00±0.00[ab]	37.25±0.25[b]
25	37.30±0.00[a]	36.45±0.45[a]	37.55±0.05[a]	36.85±0.05[a]
28	36.80±0.00[b]	37.10±0.10[c]	36.50±0.00[a]	36.50±0.00[a]

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different (p<0.05) according to Tukey's honestly significant difference. Keys: nHSE – nanosynthesized *H. sabdarifa* Ethanol, nJCH – nanosynthesized *J. carnea* Hot water, nZOH – nanosynthesized *Z. officinale* Hot water

3.7. Effects of Administration of *H. sabdariffa* Ethanol Extract on Body Weight of Albino Rats

There was no consistent dose-dependent trend across all time points. However, the 400 mg/kg dose generally showed lower weight gain compared to other groups. All groups revealed an overall increase in weight over the 28-day period. The 100 mg/kg group exhibited the most pronounced weight gain, surpassing the control group from Day 13 onward. The 400 mg/kg group consistently showed the least weight from Day 7 onward as shown in Table 7.

3.8. Effects of Administration of *J. carnea* hot water Extract on Body Weight of Albino Rats

The result in Table 8, revealed that there was no consistent dose-dependent trend across all time points. However, the 200 mg/kg dose generally showed higher weight gain, especially in the latter half of the study. The extract appears to affect weight gain differently at various doses. The 200 mg/kg dose promotes weight gain more than the control, which could be beneficial or concerning depending on the context.

3.9. Effects of Administration of *Z. officinale* hot Water Extract on Body Weight of Albino Rats

All groups, including the control, showed an overall increase in body weight over the 28-day period, indicating normal growth. The 100 mg/kg group showed the least weight gain, often lower than the control. The 200 mg/kg group consistently showed the highest weight gain among treatment groups. The control group showed steady weight gain, ending with the second-highest weight by Day 28 as shown in Table 9.

3.10. Effects of Administration of Nanoparticles of *H. sabdariffa*, *J. carnea* and *Z. officinale* Extracts on Body Weight of Albino Rats

All nanoparticle extract administered groups showed higher body weights compared to the control. Treatment group nHSE group showed the highest final body weight, followed closely by nJCH and nZOH. For the first 19 days, there were no statistically significant differences between groups. Group nHSE appeared to have the most pronounced effect on weight gain, especially in the later stages of the study as shown in Table 10.

Table 7 Effects of Administration of *H. sabdariffa* Ethanol Extract on Body Weight of Wistar Rats

Days	100 mg/kg	200 mg/kg	400 mg/kg	Control
1	93.00±1.22[a]	119.25±3.20[b]	118.25±4.97[b]	98.33±0.33[a]
4	98.75±0.48[a]	113.25±1.11[b]	109.75±4.50[b]	94.67±0.33[a]
7	101.50±1.55[a]	123.25±1.25[b]	108.25±4.57[a]	100.00±0.00[a]
10	129.33±10.35[a]	125.00±3.61[a]	108.67±6.44[a]	112.33±0.33[a]
13	137.00±9.61[b]	108.67±6.44[ab]	102.67±7.54[a]	116.33±0.33[ab]
16	135.50±0.50[c]	125.00±2.00[b]	112.00±1.00[a]	120.33±0.33[b]
19	134.00±1.00[b]	133.50±8.50[b]	114.00±0.00[a]	125.00±0.58[ab]
22	148.00±0.00[d]	126.50±0.50[b]	111.50±0.50[a]	130.33±0.33[c]
25	141.50±0.50[c]	130.50±0.50[b]	110.50±0.50[a]	132.00±0.00[b]
28	142.50±0.50[c]	141.50±0.50[c]	121.50±0.50[a]	132.00±0.00[b]

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different (p<0.05) according to Tukey's honestly significant difference

Table 8 Effects of Administration of *J. carnea* hot water Extract on Body Weight of Wistar Rats

Days	100 mg/kg	200 mg/kg	400 mg/kg	Control
1	107.75±4.60^{a}	101.75±5.50^{a}	101.25±5.42^{a}	98.50±0.50^{a}
4	96.25±2.10^{a}	96.25±1.93^{a}	104.00±1.47^{a}	95.50±0.50^{a}
7	105.75±5.38^{a}	105.25±5.96^{a}	113.50±2.75^{a}	100.50±0.50^{a}
10	114.00±7.51^{a}	110.00±7.57^{a}	116.67±3.38^{a}	111.50±0.50^{a}
13	116.00±9.50^{a}	121.33±5.33^{a}	116.67±2.96^{a}	116.00±0.00^{a}
16	112.00±14.00^{a}	136.00±5.00^{a}	115.50±0.50^{a}	120.50±0.50^{a}
19	123.00±11.00^{a}	136.00±2.00^{a}	119.00±1.00^{a}	126.50±0.50^{a}
22	118.50±0.50^{a}	138.50±0.50^{c}	117.50±0.50^{a}	130.00±0.00^{b}
25	121.50±0.50^{b}	141.50±0.50^{d}	112.50±0.50^{a}	131.50±0.50^{c}
28	129.00±0.00^{b}	150.50±0.50^{d}	123.00±0.00^{a}	132.00±0.00^{c}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different ($p<0.05$) according to Tukey's Honestly Significant Difference

Table 9 Effects of Administration of *Z. officinale* hot water Extract on Body Weight of Wistar Rats

Days	100 mg/kg	200 mg/kg	400 mg/kg	Control
Day 1	108.25±4.87^{a}	116.00±7.45^{a}	107.50±3.52^{a}	98.50±0.50^{a}
Day 4	97.75±6.38^{a}	114.25±6.54^{a}	106.25±4.25^{a}	95.50±0.50^{a}
Day 7	96.50±7.41^{a}	116.25±6.02^{a}	108.00±2.89^{a}	100.50±0.50^{a}
Day 10	108.00±4.73^{a}	120.33±7.06^{a}	113.33±2.60^{a}	111.50±0.50^{a}
Day 13	115.67±3.48^{a}	126.33±8.84^{a}	118.00±2.52^{a}	116.00±0.00^{a}
Day 16	115.00±6.00^{a}	135.50±2.50^{b}	114.50±2.50^{a}	120.50±0.50^{ab}
Day 19	118.00±7.00^{a}	137.00±2.00^{a}	115.50±3.50^{a}	126.25±3.53^{a}
Day 22	118.50±0.50^{a}	148.50±0.50^{c}	118.00±0.00^{a}	130.50±0.50^{b}
Day 25	124.50±0.50^{b}	160.50±0.50^{d}	120.50±0.50^{a}	132.00±0.00^{c}
Day 28	128.50±0.50^{a}	168.00±0.00^{d}	124.50±0.50^{a}	131.50±0.50^{c}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different ($p<0.05$) according to Tukey's honestly significant difference

Table 10 Effects of Administration of Nanoparticles of *H. sabdariffa*, *J. carnea* and *Z. officinale* Extracts on Body Weight of Wistar Rats

Days	nHSE	nJCH	nZOH	Control
Day 1	102.75±5.39a	115.00±2.68a	117.25±3.90a	98.50±0.50a
Day 4	100.50±5.11a	107.75±4.19a	113.25±3.20a	95.50±0.50a
Day 7	100.50±3.30a	114.25±2.53a	111.00±9.89a	100.50±0.50a
Day 10	108.00±2.00a	117.00±3.21a	115.67±13.86a	112.50±0.50a

Day 13	109.00±6.43a	126.33±3.93a	130.00±8.54a	116.00±0.00a
Day 16	118.50±1.50a	122.50±1.50a	122.00±2.00a	120.00±0.00a
Day 19	126.00±1.00a	129.50±0.50a	126.00±1.00a	125.50±0.50a
Day 22	137.00±0.00b	136.50±0.50b	130.50±0.50a	130.00±0.00a
Day 25	141.50±0.50d	139.00±0.00c	136.50±0.50b	132.00±0.00a
Day 28	143.50±0.50c	141.00±0.00b	141.50±0.50bc	132.00±0.00a

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different ($p<0.05$) according to Tukey's honestly significant difference. Keys: nHSE – nanosynthesized *H. sabdarifa* Ethanol, nJCH – nanosynthesized *J. carnea* Hot water, nZOH – nanosynthesized *Z. officinale* Hot water

3.11. Effects of Administration of *H. sabdariffa* Ethanol Extract on Liver Biomarkers of Wistar Rats

The effects of *H. sabdariffa* ethanol extract on liver function tests in albino rats over a 28-day period is shown in Figure 1. The liver enzymes measured were AST, ALT and ALP at days 7, 14, 21, and 28. Higher doses (200 mg/kg and 400 mg/kg) generally resulted in more pronounced changes in enzyme levels compared to the 100 mg/kg dose and control. AST levels elevated at all doses compared to control, particularly at days 7 and 14. The 200 mg/kg dose showed the highest AST levels at day 7. ALT levels showed significant elevations, particularly at day 21 for all doses while, the 400 mg/kg dose resulted in the highest ALT levels at day 21.

3.12. Effects of Administration of *J. carnea* hot water Extract on Liver Biomarkers of Wistar Rats

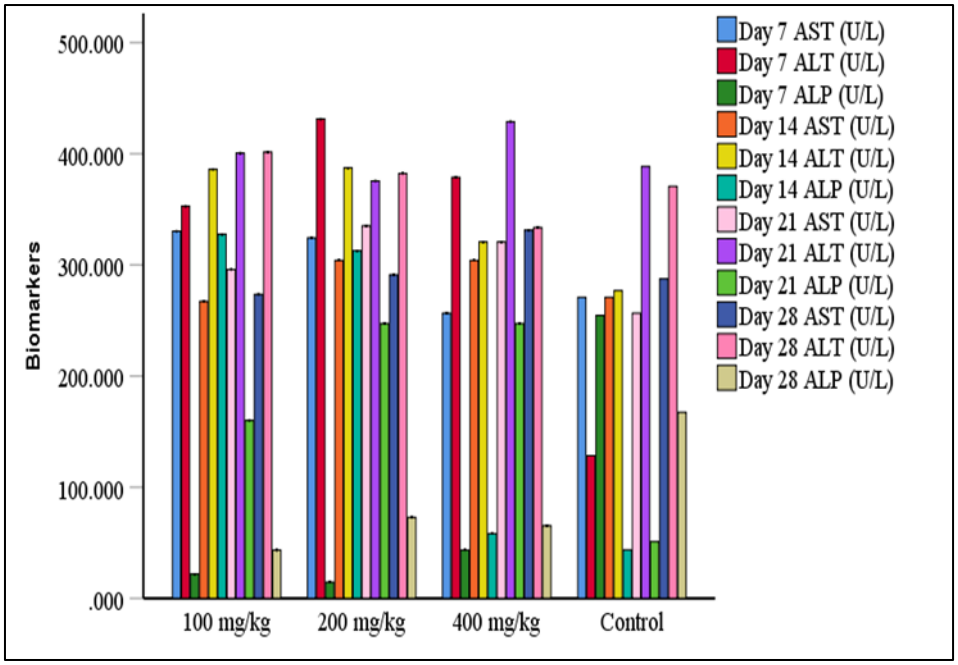
The 200 mg/kg and 400 mg/kg doses showed the highest AST levels, suggesting potential hepatocellular damage at higher concentrations. ALT levels also showed significant elevations, particularly at day 14 for the 100 mg/kg dose and day 7 for the 400 mg/kg dose. The control group maintained relatively stable enzyme levels throughout the study period as shown in Figure 2.

3.13. Effects of Administration of *Z. officinale* hot Water Extract on Liver Biomarkers of Wistar Rats

The 200 mg/kg dose often showed the most pronounced changes, particularly for ALT and AST. Enzyme levels fluctuated over time, with different patterns for each enzyme, dose, and time point. AST levels was generally elevated across all doses compared to control, with the 200 mg/kg dose showing the highest levels, particularly at day 21. ALT levels showed significant elevations, particularly for the 200 mg/kg dose at day 7. The 400 mg/kg dose also showed elevated ALT at day 7, but to a lesser extent than the 200 mg/kg dose as shown in Figure 3.

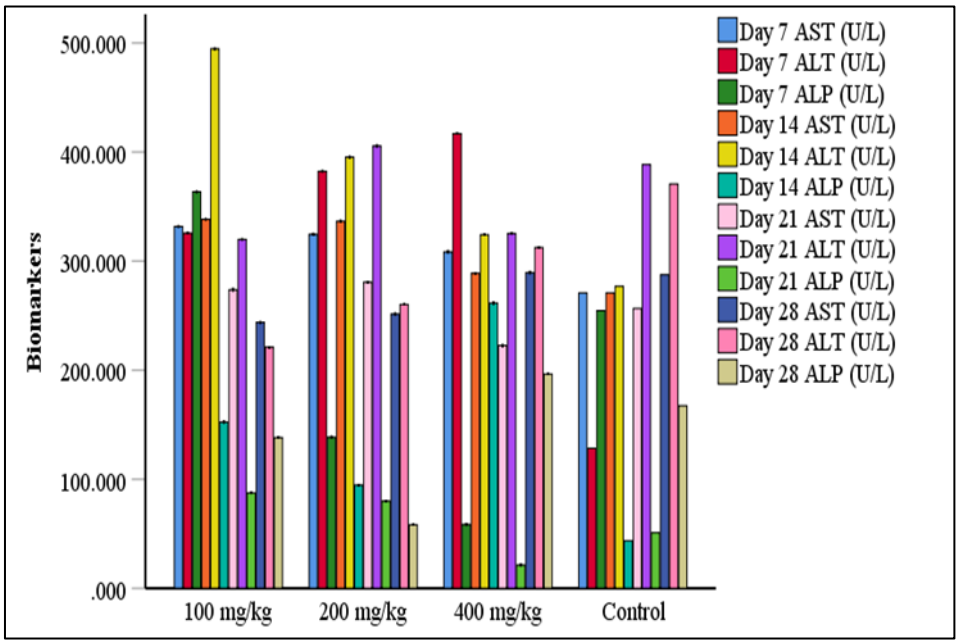
3.14. Effects of Administration of Nanoparticles of *H. sabdariffa*, *J. carnea* and *Z. officinale* Extracts on Liver Biomarkers of Wistar Rats

Higher doses of 100 mg/kg and 400 mg/kg resulted in more pronounced changes in enzyme levels compared to the 100 mg/kg dose and control. AST was elevated at all doses compared to control, particularly at days 7 and 14. The 200 mg/kg dose showed the highest AST levels, especially at day 7. ALT showed significant elevations, particularly at day 21 for all doses while, 400 mg/kg dose resulted in the highest ALT levels at day 21. ALP demonstrated less changes compared to AST and ALT. Some elevation were observed in the enzyme levels at day 14 for the 100 mg/kg and 200 mg/kg doses with little to no changes in the control group as shown in Figure 4.



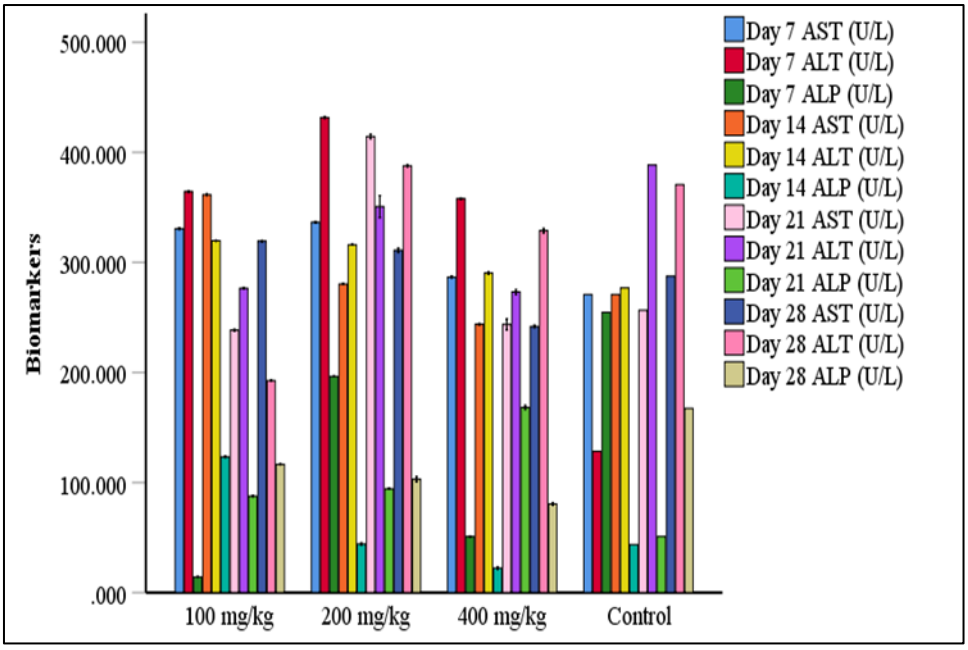
Keys: Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP)

Figure 1 Effects of Administration of *Hibiscus sabdariffa* Ethanol Extract on Liver Biomarkers of Albino Rats



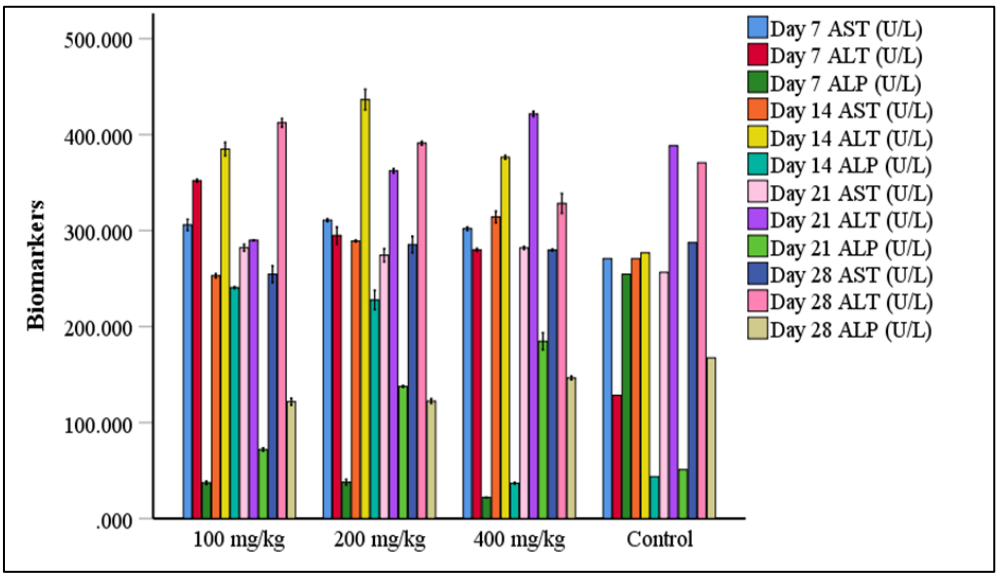
Keys: Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP)

Figure 2 Effects of Administration of *Justicia carnea* hot water Extract on Liver Biomarkers of Albino Rats



Keys: Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP)

Figure 3 Effects of Administration of *Zingiber officinale* hot Water Extract on Liver Biomarkers of Albino Rats



Keys: Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP)

Figure 4 Effects of Administration of Nanoparticles of *H. sabdariffa*, *J. carnea* and *Z. officinale* Extracts on Liver Biomarkers of Albino Rats

3.15. Effect of Administration of Plant Extracts and Honey on Wistar Rat's Haematological Parameters

Extracts administration showed the highest increase in the WBC (16.50), negative control drastically reduced WBC (1.30), confirming its immunosuppressive action as shown in Table 11. Extracts in combination with honey maintained elevated WBC (6.88) compared to vehicle, indicating sustained immune activation. Lymphocytes (LYM) increased significantly with extracts (57.00%) while, extracts with honey increased to 60.50%. Neutrophils (NEU) decreased in all treatments compared to vehicle, possibly due to increased production of lymphocyte. Extracts and extracts - honey increased RBC count, hemoglobin (HGB), and hematocrit (HCT). Honey alone showed lower RBC-related values. Extracts only and positive control dramatically increased platelet count (1643.50 and 1374.50). Red cell distribution width

(RDW-SD and RDW-CV) showed minor variations and erythrocyte sedimentation rate (ESR) was reduced in extracts only and honey only groups, potentially indicating reduced inflammation.

Table 11 Effect of Administration with Plant Extracts and Honey on Wistar Rat's Haematological Parameters

Haematological indices	Vehicle	Extracts only	Positive control	Negative control	Honey only	Extracts + Honey
WBC (109/L)	8.30±0.00c	16.50±0.50d	6.50±0.50b	1.30±0.10a	2.45±0.05a	6.88±0.03bc
NEU (%)	51.50±0.50d	37.00±1.00c	33.50±0.50a	47.50±0.50c	41.00±1.00b	35.70±0.30b
EOS (%)	2.00±0.00b	2.00±0.00b	4.00±0.00c	2.00±0.00b	2.00±0.00b	0.00±0.00a
BAS (%)	2.00±0.00b	0.00±0.00a	2.00±0.00b	2.00±0.00b	0.00±0.00a	0.00±0.00a
LYM (%)	41.00±1.00a	57.00±1.00bc	54.50±0.50b	45.50±0.50a	55.50±0.50bc	60.50±1.50c
MON (%)	2.00±0.00a	2.00±0.00a	6.00±0.00b	2.00±0.00a	2.00±0.00a	2.00±0.00a
RBC (1012/L)	6.19±0.02c	6.67±0.03d	5.25±0.01b	7.35±0.01e	2.09±0.01a	7.64±0.01f
HGB (g/L)	10.60±0.10c	13.15±0.15d	9.90±0.10b	14.10±0.10e	3.45±0.05a	14.65±0.05e
HCT (%)	40.30±0.30c	41.25±0.25c	31.45±0.05b	44.60±0.20d	12.10±0.10a	49.45±0.05e
MCV (fL)	65.85±0.05d	61.85±0.15c	59.45±0.45ab	60.45±0.05bc	58.30±0.10a	65.25±0.55d
MCH (pg)	17.20±0.20a	20.05±0.05c	18.60±0.10b	19.15±0.15bc	16.35±0.25a	19.30±0.20bc
MCHC (g/L)	265.50±1.50a	322.50±2.50c	311.00±1.00c	322.50±2.50c	282.50±2.50b	292.50±2.50b
RDW-SD	24.70±0.00d	24.00±0.00c	21.60±0.10b	23.60±0.10c	17.50±0.10a	25.70±0.10e
RDW-CV (%)	15.00±0.00c	15.60±0.00d	14.15±0.15b	15.75±0.05d	11.95±0.05a	15.75±0.05d
PLT (103/L)	448.50±0.50a	1643.50±3.50e	1374.50±4.50d	707.50±1.50b	438.50±0.50a	977.50±1.50c
MPV (fL)	6.83±0.03d	6.80±0.00cd	6.50±0.00ab	6.40±0.00a	7.15±0.05e	6.65±0.05bc
PDW (%)	8.20±0.00cd	8.20±0.00cd	8.10±0.00bc	7.65±0.05a	8.35±0.05d	8.00±0.00b
P-LCR (%)	8.35±0.05d	8.93±0.03e	7.23±0.03b	5.73±0.03a	10.93±0.03f	7.65±0.05c
PCT (%)	0.31±0.00a	1.12±0.02e	0.93±0.03d	0.43±0.03b	0.32±0.01a	0.66±0.01c
RCDW (%)	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
LCDW (%)	0.13±0.03b	0.00±0.00a	0.13±0.03b	0.10±0.00b	0.22±0.02c	0.10±0.00b
ESR (mm/Hr)	1.00±0.00	0.00±0.00	1.00±0.00	1.00±0.00	0.00±0.00	1.00±0.00

Key: A- Vehicle, B- Extracts only, C- Positive control, D- Negative control, E- Honey only, F- Extracts + Honey

4. Discussion

The study evaluated the safety and immunomodulatory potential of crude and nanosynthesized extracts from *H. sabdariffa*, *J. carnea*, and *Z. officinale* in Wistar rats, revealing generally low toxicity profiles with some dose-dependent effects on body temperature, weight, organ weights, liver biomarkers, and hematological parameters. Behavioral observations in acute toxicity aligned with OECD guidelines, showing no mortality or severe changes up to 400 mg/kg, consistent with prior acute toxicity studies of *H. sabdariffa* extracts in rats, which reported no adverse effects at doses up to 5000 mg/kg [22, 27]. However, transient sedation observed here contrasts with minimal behavioral alterations in *Z. officinale* acute studies, suggesting species-specific responses [28].

Sub-chronic exposure revealed fluctuations in body temperature without clear dose-dependence, differing from elevated temperatures in sub-chronic ginger oil studies in rats [29]. Body weight changes varied: *H. sabdariffa* at 400 mg/kg suppressed gain, akin to dose-dependent inhibition in sub-chronic toxicity of *H. sabdariffa* calyces [30], while *J. carnea* at 200 mg/kg promoted gain, supporting its hematopoietic effects in anemic rats [6]. Nanoparticles accelerated

weight gain, potentially due to enhanced bioavailability, as seen in green-synthesized AgNPs from plant extracts improving metabolic parameters [15].

Organ weight alterations indicated potential toxicity while increased liver weights across extracts suggest hepatocellular hypertrophy, comparable to sub-chronic *H. sabdariffa* studies showing mild hepatotoxicity [30]. Kidney decreases in *J. carnea* align with nephroprotective yet dose-dependent effects in other herbal sub-chronic assays [23]. Liver biomarkers (AST, ALT, ALP) elevations at higher doses indicate hepatotoxicity, mirroring sub-chronic *Z. officinale* extract effects in rats [24]. Nanoparticles showed similar patterns, but milder, contrasting with enhanced toxicity in some AgNPs studies due to oxidative stress [16].

Hematological improvements like increased RBC and platelets support immunomodulatory roles and is consistent with *J. carnea*'s hematopoietic activity in phenylhydrazine-induced anemia [6]. Combined with honey, extracts enhanced parameters, aligning with honey's immunomodulatory effects in infected models [26].

Overall, findings corroborate low toxicity in acute settings but highlight sub-chronic risks at higher doses, differing from non-toxic profiles in shorter *H. sabdariffa* assays [30]. Nanoparticles potentiated efficacy with minimal added toxicity, supporting green synthesis advantages [15].

5. Conclusion

The crude and nanosynthesized extracts exhibit low acute toxicity and promising immunomodulatory and hematopoietic effects, particularly when combined with honey, highlighting their therapeutic potential against uropathogen infections. However, sub-chronic elevations in liver enzymes warrant caution at higher doses.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no competing interests

Statement of ethical approval

Ethical approval for the use of laboratory animals was granted by the Research ethical Committee of the Centre for Research and Development (CERAD), Federal University of Technology, Akure, Nigeria.

Author contributions

MKO: conceptualization, methodology, writing-original draft, supervision. MI: plant identification, review. IAA: chemical analysis, docking. BOO: extraction, characterization. REB: methodology. ETA: data analysis, editing. All authors approved the final manuscript.

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