Hyperlipidemic and Leucopenia Effects of The Ethanolic Leaf Extract of *Parquetina Nigrescens* in Wistar Rats Exposed to Phenylhydrazine

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Abstract

Introduction: The huge reliance on medicinal plants in Africa can be attributed to cultural background, low economic levels and poor access to orthodox medications. However, often times, such reliance are accompanied by the challenges of poor evaluation of their safety profiles before commercialization. The present study investigated the effect of ethanolic leaf extract of *Parquetina nigrescens* (ELEPN) on lipid profile and white blood cell parameters in male Wistar rats, exposed to Phenylhydrazine.

Materials and methods: In the current investigation, 30 male Wistar rats weighing between 138g and 235g each were employed. They were divided into six (6) groups of five rats each, with group 1 (control) being untreated normal rats, group II receiving 400 mg/kg of ELEPN treatment, group III receiving phenylhydrazine (P-H) treatment only, group IV receiving 400 mg/kg of ELEPN + P-H treatment, group V receiving 800 mg/kg of ELEPN treatment, and group VI receiving 800 mg/kg of ELEPN + P-H treatment. After treating the respective groups with the specified treatments for 21 consecutive days, 5ml of blood samples were harvested from each of the study animal via cardiac puncture.

Results: The result revealed that the 400mg/kg and 800mg/kg doses of the ELEPN had remarkable ($p<0.05$) elevations in TC, TG, LDL-C, VLDL and HDL-C levels in a dose-dependent manner of the extract, in both phenylhydrazine exposed and unexposed male Wistar rats. Again, there was a dose-dependent decrease in lymphocyte, Eosinophil and Monocyte. There was an increase in the concentration of Neutrophils and no change in the concentration of Basophil.

Conclusion: The present study recorded a significantly elevated levels of the lipid profile and a decrease in the white blood cell (WBC) differentials in all ELEPN treated rats. The extract may therefore have the potential of causing hyperlipidaemia and leucopenia.

Keywords: Medicinal, lipid profile, leucopenia, hyperlipidaemia and white blood cell.

Introduction

Hyperlipidemia, characterized by elevated levels of lipids in the blood, is a well-documented risk factor for cardiovascular diseases, which remain a leading cause of morbidity and mortality worldwide. Meanwhile, leucopenia, a condition characterized by a decrease in white blood cell count, can compromise the immune system's ability to defend against infections and is a concerning health issue. Both hyperlipidemia and leucopenia can arise due to various factors, including exposure to chemical agents. Phenylhydrazine, a chemical compound frequently used in laboratory experiments, has been shown to induce oxidative stress and disrupt hematological parameters in experimental animals. Understanding the potential modulatory effects of natural compounds on phenylhydrazine-induced hyperlipidemia and leucopenia is essential, as it may provide valuable insights into novel therapeutic strategies

Plants serve different purposes such as medicinal, food, forestry, building and furniture construction, ornaments, etc. plants also increase the oxygen content of the environment by releasing oxygen into the atmosphere as a byproduct during photosynthesis **(Tapsell *et al.*, 2006; Zabbey et al., 2022).** Medicinal plants are known to be endowed with helpful attributes or better still produce beneficial therapeutic impacts the body. They are the mainstay of the traditional medicine and ethnomedicinal exploitations all over the world. In fact, record has it that, well over eighty percent of the global population continue to depend majorly on herbal or ethnomedicine for the management of diseases/ailments, typically in developing countries **(Ekor, 2014).** In traditional African medicine and various cultures across the world, medicinal plants have served as the foundation for the treatment of many conditions. It has been a marked source of active compounds used as pharmacological lead compounds. The history of ethnomedicine or herbs is as old as human history, and early people used plants to tang and treat ailments **(Tapsell et al 2006).**

In our locale, one very important medicinal plant commonly adopted in tradomedicinal practices is *Parquetina nigrescens* (*P. nigrescens or P. nigrescens*). *It* has been reported to be a medicinal plant capable of preventing or ameliorating inflammation, improve blood indices and has analgesic property **(Owoyele *et al.*, 2009).** Different portions of it have been said to depress the lipid oxidation in rats liver by hindering oxidative species **(Ayoola *et al.*, 2011).**

P. nigrescens, have been identified as a very useful medicinal plants with wide applications. *P. nigrescens* or bullock, as it is also called, is a hedge plant common in equatorial West Africa, and has been used in traditional medical practice for years. Both the roots, leaves and latex are used for medical purposes.

P. nigrescens is a persistent shrub, with pairing stem and arboreal base shortly conical with a smooth long stem on the leaves. In a previous study, *P. nigrescens* was described to have haematinic attributes or activities; thus increasing red blood cell in experimentally induced anaemic conditions in rats in a graded dose manner **(Saba et al., 2010).** More so, in Nigeria herbal preparation made from *P. nigrescens*, S*orghumbilolor moench and Harungana madagascariensis Lameex poir,* are locally employed in the treatment of anaemia **(Gbadamosi et al., 2012).**

This study was carried out to determine the effect of ethanolic leaf extract of *P. nigrescens* (ELEPN) on the lipid profile and white blood cell parameters in male Wistar rats, exposed to Phenylhydrazine.

Materials and methods

The fresh leaves of *Parquetina nigrescens* (*P. nigrescens)* were obtained from a private farm in Oje Community, Ibadan South East Local Government, Oyo State, Nigeria. The plant was identified and authenticated by Dr C. Ekeke of the University of Port Harcourt herbarium, Rivers State, Nigeria. The *parquetina nigresens* was plucked off from the stem, washed under running tap water and air-dried under a shed. After about two weeks of air-drying, it was grinded into powder by a milling machine (Corolla, China). Over 175g of the fine powder was obtained after pulverizing. The phytochemical analysis was carried out using standard procedures as described by Harbone (1998) and AOAC (1990). About 50g of the powdery form of the PN plant was weighed and put into a maceration jar. Thereafter, 1000ml of ethanol was added into the maceration jar and left for 24 hours. With intermittent shaking, it was filtered using washman filter paper. The filtrate was concentrated using rotary evaporator and then further dispensed into the evaporation dishes and placed on water bath set at the temperature of 65^{o}C. More extract was added intermittently until the desired paste of the extract was achieved. Thereafter, the ethanolic extract of P.N was removed and put into clean universal bottles and refrigerated at the temperature of 2 – 8^{o}C, from where it was then used for the study.

A total of thirty male Wistar rats weighing between 138g and 235g were acquired and accommodated in the animal house of Faculty of Basic Medical Sciences (FBMS), University of Port Harcourt and kept under the natural day light and night cycles and fed with feeds and water ad-libitum. The study animals were allowed fourteen days of acclimatization to their new handling and environment prior to the commencement of experimental procedures on them. The experimental rats were thereafter divided into six (6) groups of five animals each and was labeled group 1, group II, group III, group IV, group V and group VI. Haemotoxcity was induced using Phenylhydrazine in groups 11, V and VI, based on their body weights for two consecutive days after which treatments with the varying doses of the extract commenced and lasted for twenty-one days.

The experimental animals were given varying concentrations of the extracts as shown below:

Group I: Negative control (fed with only rat feed and distilled water) 1ml distilled water for 21 days.

Group II: Positive Control (Animals of this group were given only 0.7mls/kg of P-H for two days retroperitoneally).

Group III: Test group 1(animals of this group were given 400mg/kgbw of *P. nigrescens* extract alone orally for 21 days).

Group IV: Test group 2 (Animals of this group was given 800mg/kgbw of parquetina extract orally alone for 21 days).

Group V: Test group 3 (animals of this group were given both 0.7mls of phenylhydrazine and 400mg/kgbw of *P. nigrescens* for 21 days).

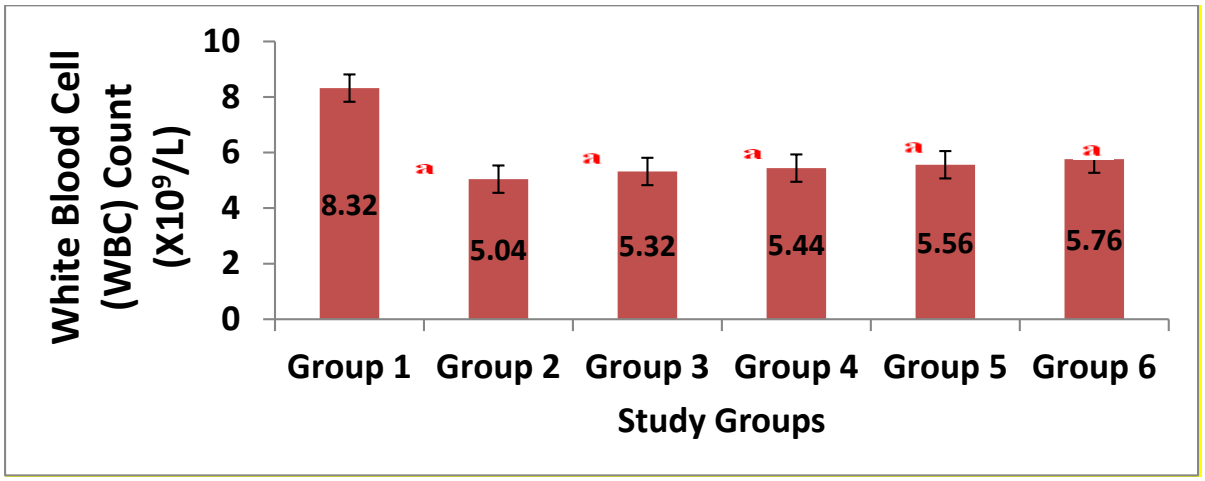
Group VI: Test group 4 (animals of the group were given both 0.7mls of phenylhydrazine and 800mg/kgbw of *P. nigrescens* extract for 21 days.

At the end of the treatment interval, the experimental animals were scarified by placing each of them in a container containing cotton wool soaked with chloroform for sedation. Thereafter the rats were laid on a dissecting board in a supine position and dissecting pins were used to fasten their limbs to the board. Using cardiac puncture, blood samples were collected with the lithium – heparin

anticoagulant tubes. The laboratory analyses of the white blood cell differentials were done using microhematocrit method while Lipid profile analysis was done using VIS spectrophotometer (S23A Helmreasinin model).
The quantitative data obtained from the present study were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0. Statistical significance was determined using a one-way analysis of variance (ANOVA) and Post-Hoc multiple comparison and $p< 0.05$ was considered statistically significant. The values were expressed as mean ± standard error of mean (SEM).
Ethical approval for study was sought and obtained from the Ethics Committee of the Centre for Research Management and Development, University of Port Harcourt, Nigeria.

Results and Discussion

Results



Values represent mean ± SEM, n=5; [a] significant at p<0.05 compared to Group 1; [b] significant at p<0.05 when compared to group 2.

Figure 1: Effect of administration of ELEPN on WBC levels in Phenylalanine exposed male Wistar rats.

Figures 1 above shows the result of the effect of administration of ELEPN on white blood count (WBC) in phenylhydrazine exposed male Wistar rats. The white blood cell count (WBC) of the study animals showed a uniform variation as all the treated groups (group II, III, IV, V and VI), irrespective of whether treated with only ELEPN or ELEPN + P-H were significantly ($P < 0.05$) reduced when compared to group 1.

-Table 1: Effects of administration of ELEPN on WBC Differentials in Phenylhydrazine exposed male Wistar

Group and Treatment	Neutrophil (%)	Lymphocyte (%)	Eosinophil (%)	Basophil (%)	Monocyte (%)
Group 1: Control	23.80 ± 1.93	71.20 ± 1.24	1.00 ± 0.45	0.00 ± 0.00	4.00 ± 1.30
Group II: P-H only	24.40 ± 2.38	70.20 ± 1.13	0.20 ± 0.20	0.00 ± 0.00	2.40 ± 0.51

Group III: 400mg/kg ELEPN	31.20 ± 1.83	66.80 ± 1.71	0.60 ± 0.24	0.00 ± 0.00	1.40 ± 0.68 [a]
Group IV: 800mg/kg ELEPN	26.80 ± 5.05	60.20 ± 1.69	0.40 ± 0.13	0.00 ± 0.00	1.20 ± 0.51
Group V: 400mg/kg ELEPN + P-H	26.40 ± 5.90	63.80 ± 1.35	0.40 ± 0.05	0.00 ± 0.00	1.30 ± 0.08
Group VI: 800mg/kg ELEPN + P-H	35.20 ± 2.82[b]	65.40 ± 2.82	0.50 ± 0.10	0.00 ± 0.00	1.40 ± 0.10[b]

Values represent mean ± SEM, n=5; [a] significant at p<0.05 compared to Group 1; [b] significant at p<0.05 when compared to group 2.

Table 2: Effect of administration of ELEPN on Lipid Profile in Phenylhydrazine exposed male Wistar rats

Group and Treatment	TC (mmol/L)	TG (mmol/L)	HDL (mmol/ L)	LDL (mmol/L)	VLDL (mmol/L)
Group 1: Control	4.44 ± 0.17	2.53 ± 0.43	2.19 ± 0.07	6.63 ± 0.25	3.09 ± 0.44
Group II: 0.7 mls P-H only	2.60 ± 0.19 [a]	1.88 ± 0.22 [a]	1.58 ± 0.56 [a]	3.96 ± 0.39 [a]	1.16 ± 0.18[a]
Group III: 400mg/kg ELEPN	7.32 ± 0.75 [a]	6.07 ± 0.58 [a]	7.34 ± 0.39 [a]	10.79 ± 0.74 [a]	5.59 ± 0.31
Group IV: 800mg/kg ELEPN	7.72 ± 0.26 [b]	6.34 ± 0.76[b]	7.62± 0.69[b]	11.25 ± 1.38 [b]	6.42 ± 0.03[b]

Group V: 400mg/kg ELEPN + P-H	7.12 ± 0.32 [a]	5.65 ± 0.81 [a]	6.22 ± 0.63[a]	9.80 ± 0.69 [a]	5.33 ± 0.18 [a]
Group VI: 800mg/kg ELEPN + P-H	7.62 ± 0.54 [b]	6.16 ± 0.61[b]	7.28 ± 1.00[b]	10.87 ± 0.84 [b]	6.13 ± 0.22 [b]

Values represent mean ± SEM, n=5; **[a]** significant at $p<0.05$ compared to Group 1; **[b]** significant at $p<0.05$ when compared to group 2.

Table 2 above shows the result of the effect of administration of ELEPN on lipid profile in phenylhydrazine exposed male Wistar rats. There was a significant decrease in TC, TG, LDL-C, HDL-C and VLDL all in group II, with respect to group 1. There was also a significant increase in all the lipid profiles in III and IV, with respect to group I. Finally, when comparing groups V and VI with group II, it was found that all the lipid profiles were significantly higher in groups IV and VI than in group II.

Discussion

Effects of administration of the ELEPN on some WBC differentials in male Wistar rats

Table 1 above shows the result of the effect of administration of ELEPN on white blood cell differentials in phenylhydrazine exposed male Wistar rats. There was an increase in neutrophil count, and a decrease in the lymphocyte count, Eosinophil count, Basophil count and Monocyte count in group 2, when compared with the group 1 (the negative control group). However, only the decrease in monocyte was found to be statistically significant. Again, there was found a dose dependent but statistically insignificant increase in neutrophils and lymphocytes in groups III and IV with respect to group 1 while there was a decrease in the other differentials in the same groups with respect to group 1. Finally, when comparing groups V and VI with group II, there was found a significant increase in neutrophil and monocytes only in group VI, with respect to group II.

This result is similar to the earlier finding of **Owoleye et al., (2011).**

Neutrophils are a type of white blood cell that plays a crucial role in the body's immune response, particularly in fighting bacterial infections. Various factors can lead to an increase in neutrophil counts, including infection, inflammation, medications, and certain medical conditions. The extract-induced increase in neutrophil counts could suggest a potential immunomodulatory effect. It may therefore be worth exploring whether the extract can influence the immune system in a beneficial or detrimental way

Apart from the effect on neutrophils, there was found a decrease in the other differentials in groups III and IV with respect to Group I. This shows that the extract may exhibit the tendency of causing a decrease in white blood cell differentials. The specific mechanism by which the extract causes leukopenia may be via its chemical composition and how it interacts with the immune system and bone marrow **(Pedrosa et al., 2018).** Surprisingly, apart from the significantly increased and decreased neutrophils and lymphocytes levels respectively by the 800mg/kg dose of ELEPN, there were no significant changes in the rest of the WBC differentials of all threated rats. The leucopenia caused by ELEPN is being implicated in increased susceptibility to infections, impaired wound healing, increased risk of secondary complications, Fatigue and weakness and haematological disorders. Leucopenia can weaken the immune system, making individuals more susceptible to bacterial, viral, and fungal infections. With a reduced number of white blood cells, the body's ability to defend against pathogens is compromised. Leucopenia can increase the risk of developing secondary complications, such as sepsis, as the body struggles to control and eliminate infections. Also, the decrease in white blood cells can lead to symptoms such as fatigue, weakness, and overall decreased energy levels. This can significantly impact the individual's quality of life. Prolonged or severe leucopenia may also be associated with other hematological disorders, such as anemia or thrombocytopenia (reduced platelet count), further complicating the individual's health **(Omale et al.,2013).** The extract may have mediated its action via Myelosuppression, Apoptosis, immune System modulation, disruption of Hematopoiesis, bone Marrow Damage or autoimmune Reaction **(Wang et al., 2018).**

Effects of administration of the ELEPN on lipid profile in male Wistar rats

The data presented in Table 2 shows the result of the effect of administration of ELEPN on some lipid profile parameters in phenylhydrazine (P-H) exposed male Wistar rats. The levels of total cholesterol (TC) in the studied animals were seen to be significantly ($p< 0.05$) increased in groups II, IV, V and VI when compared to that of group 1 (control group). Surprisingly though, the phenylhydrazine-only-treated group (i.e., group 3) indicated a significant ($p < 0.05$) reduction in the TC level when compared to those of groups 1, 2, 4, 5 and 6. Meanwhile, the P-H + 800mg/kg ELENPN treated group 5 animals manifested the highest significantly ($p<0.05$) raised level of TC when compared to those of all other groups. Considering the changes in triglyceride (TG) levels following the administration of the ELEPN, both the 400 and 800mg/kg ELEPN and then the 800mg/kg ELEPN + P-H (i.e. groups II, V and VI) had significantly elevated levels when compared to those of control and P-H only-treated groups (i.e. groups 1 and II). It is important to note that the P-H only-treated group had the lowest TG level even when compared to that of the untreated rats (in group1) which was though not marked ($p>0.05$).

When the changes in the level of high-density lipid cholesterol (HDL-C) was considered; all the treated animals showed markedly ($P < 0.05$) increased HDL-C levels when compared to group 1 except group 3 which served as a positive control. It is important to note that, despite the possible toxicity effects of phenylhydrazine (P-H) in group 6, the ELEPN administration was able to significantly ($P<0.0$) improve HDL-C level.

The findings on the level of low-density lipid cholesterol (LDL-C) revealed that the 400 and 800mg/kg of ELEPN alone treated groups (II and V) had not only significantly ($p<0.05$) increased LDL-C levels when compared to control group, but the highest value amongst other treated groups (and in a dose-pendent fashion). It is noteworthy that the P-H only treated group (group III) was the only group with markedly ($p<0.05$) decreased LDL-C level when compared to the control group. Similarly, the LDL-C values of the 400mg/kg ELEPN + P-H and 800mg/kg ELEPN + P-H treated groups (i.e. groups 4 and 8) did not show any significant changes when compared to that of control group.

The value of very low-density lipid (VLDL) in groups II, III, IV and VI did not vary marked ($p>0.05$) when vary greatly as only group 5 was markedly ($P < 0.05$) when compared to that of the control group. Group III (i.e. P-H only treated group) had the lowest marked ($p<0.05$) level of VLDL when compared to the rest of the other treated groups.

This result corroborates that done by **Owoyele *et al.*, (2011),** which showed decreased white blood cell count and a significant upsurge in TC, LDL-C, with no change in high density lipoprotein.

The outcome of the present study is also consistent with the finding of Owoyele et al., (2011), who used 50 mg/kg, 100 mg/kg and 150mg/kg of the aqueous root extract of the plant in same rat models

However, the results contradict that earlier submitted by **Ojuade *et al.*, (2021),** which used 200, 400 and 800 mg/kg of aqueous extract of the whole plant of P. nigrescens and reported an antihyperlipidemic activity of the plant in streptozotocin–nicotinamide-induced type 2 diabetic rats.

The foregoing therefore reveals the need for a complete profiling of the various portions (leaf, stem and root) of *P. nigrescens* plant as to better understand their specific or variant biological effects, particularly on lipid profile of a typical mammalian model like Wistar rats.

Still considering the result of the present study on lipid profile, it was surprising to note that phenylhydrazine (P-H), which is known to have several adverse toxicity effect on single exposure via the oral route in experimental models, was seen to be likely exerting regulatory effects (with beneficial levels of TC, TG, LDL-C and VLDL). The possible mechanism of action on these parameters by the phenylhydrazine is not clearly understood here and that, this outcome is contrary to some earlier reports **(Owoyele *et al.*, 2011),** which submitted phenyl hydrazine's hypolipidaemic effects in rats. A specific determination of the actual effect and prediction of the possible mechanism of action of phenylhydrazine on lipid profile in rats by further studies may be helpful its precise future application in biomedical researches.

Conclusions

Since elevated levels of TC, TG, LDL-C, and VLDL are typically associated with an increased risk of atherosclerosis and cardiovascular diseases, caution should be taken in the consumption of the extract as these lipids are known to contribute to the buildup of plaques in arteries. Again, the decrease in differential white blood cell count caused by the extract shows its likely immunosuppressive effect.

Consent

It is not applicable.

Ethical Approval

The ethical approval to carry out this work was sought for and obtained from the ethics and research committee of the University of Port Harcourt and preserved by the author(s).

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Competing interests

Authors have declared that no competing interests exist.

Authors' contributions

Author Ojeka, S.O, conceived the study, designed the protocol and contributed in the manuscript writing while author Nkwadochi, D.O coordinated the experiment, carried out the laboratory procedures. Finally, author Zabbey, V. Z performed the statistical analysis and data interpretation. All authors read through and approved the final manuscript.

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