Safety Evaluation of Ethanolic Extract of *Parquetina nigrescens* Leaves (Afzel.) on Selected Tissues in Letrozole-induced Polycystic Ovarian Syndrome Rats

Femi-Olabisi, Fehintoluwa Joy*[1], Komolafe, Marvellous Olaoluwa[1], Oladoye, Odunayo[2] and Alabi, Toluwalope Susan[1]

[1]*Biochemistry Unit, Department of Biological Sciences, Mountain Top University, Ogun, Nigeria.*
[2]*Department of Biochemistry, Unversity of Ilorin, Ilorin. Nigeria*.
**Corresponding Author's Email: Fehintoluwao@yahoo.com*

Abstract

The toxicological effects of ethanolic extract of Parquetina nigrescens leaves (EEPNL) on letrozole-induced polycystic ovarian syndrome (PCOS) in Wistar rats were evaluated. Twenty female Wistar rats (170.81 ± 5.25g) were randomly assigned into 5 groups (A - E) of four animals each. Animals in group A received 1 ml of distilled water and group B-E received 1mg/kg body weight of letrozole daily basis for a period of 21 days orally. The letrozole-treated groups, B-E, were then administered 1 ml of distilled water, co-administration of 7.14mg/kg of metformin and 2mg/kg clomiphene citrate (reference drug), 50 mg/kg b.wt of EEPNL and 100 mg/kg b.wt of EEPNL respectively for a period of 14 days. The animals were sacrificed 24 hours after the last treatment dose and the blood obtained via jugular puncturing was used in determining some toxicological indices such as liver function indices (albumin, bilirubin, globulin and total protein), kidney function indices (Urea, uric acid and creatinine), and enzyme assay (Alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase). The result revealed mild alterations in the parameters under studied in this work and this suggests that the ethanolic leaf extract of Parquetina nigrescens at doses investigated may be safe when used in the treatment of PCOS.

Keywords*: Letrozole, Safety profile, Parquetinanigrescens, Polycystic Ovarian Syndrome, Rat,*

1.0 Introduction

Polycystic ovary syndrome is one of the most common endocrine disorders affecting approximately 5-7% of reproductive age (Nagarathna, 2014), and also constitute as the most prevalent cause of infertility in women. It was unraveled by Michael Leventhal and Irving Stein in 1935 when they established the connection in patients with amenorrhea, infertility and enlarged multicystic ovaries. *(*Azziz *et al.*, 2004). There have been various metabolic disorders that have been associated with PCOS such as hypertension, dyslipidemia and obesity and metabolic syndrome. Wild et al. 2002 reported a spike in the level of these mentioned disorders compared with non-PCOS women. Studies have also suggested the most probable etiology of PCOS might be through insulin resistance and the treatment approach to PCOS will involve the use of insulin sensitizers such as clomiphene citrate and metformin (Shivali *et al*., 2018).

The usage of plant for therapeutic purpose has been widely acclaimed all over the world and those having medicinal purposes are called medicinal plant. Medicinal plants are important elements of various indigenous medical systems in all countries of the world. Various plants with anti-obesity and hypoglycaemic activities have been used in the management of PCOS. A very good example of this is the *Parquetina nigrescens.*

Parquetina nigrescens (Afzel) also known as Mgbidim Gbe (Igbo), Kwankwanin (Hausa), ewe Ogbo (Yoruba) is a common plant found in West African countries such as Ghana and Nigeria (Femi-olabisi *et al.,* 2020). The leaves has been reported to inhibits inflammation, and also elicit analgesic property (Sopeyin *et al* 2016). It has been used in the treatment of menstrual disorders (Femi-olabisi *et al* 2022). Extract in the aqueous form and isolated compounds from *Parquetina nigrescens* have been evaluated in both *in-vivo* and *in-vitro* assays for PCOS treatment potentials (Paul *et al*., 2018). Therefore, the present study was aimed at investigating the safety/toxicity risk of ethanolic extract of *Parquetina nigrescens leaves* (EEPNL) on letrozole-induced polycystic ovarian syndrome in female rats.

2.0 Material and Methods

2.1 Plant Material

Fresh leaves of *Parquetina nirescens* were obtained from the premises of Mountain of Fire and Miracles Ministries, Ibafo, Ogun State, Nigeria. The plant was authenticated at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. A voucher specimen UH 001/0980 was deposited at the Herbarium.

2.2 Animals

Twenty female albino rats (*Rattus novergieus*) with an average weight of (170.81 ± 5.25g) were obtained from the animal holding unit of the Mountain Top University, Ogun State, Nigeria. The animals were kept in a well-ventilated house condition (temperature: 22±3^{o}C; photoperiod: 12h/12h light/dark cycle; humidity: 45-50 %) and fed with rat pellets and water.

2.3 Drugs, Assays Kits, and Chemicals

Albumin, bilirubin, aspartate aminotransferase (AST), alkaline phosphatase, alanine aminotransferase (ALT), urea, uric acid, creatinine and total protein assay kits were products of Randox laboratory, Liquizyme, United Kingdom.

2.4 Preparation of Plant Extract

Fresh leaves of *Parquetina nigrescens* (1.01 kg) were washed, air-dried to attain a constant weight and pulverized with an electric blender (Kenwood Ltd, Havant, United Kingdom). The powdered material was weighed (500 g) and soaked in 3L of absolute (concentrated) ethanol (Sigma-Aldrich, Burlington, USA) for 48hrs. The extract was sieved and filtered with Whatman's No. 1 filter paper (Whatman International Ltd. Maidstone, England). The filtrate was subjected to the rotatory

evaporator to concentrate the plant sample. The filtrate was lyophilized to give 544.17g resultant yield of 13.2%.

2.5 Animal grouping and extract administration

A total of 20 female rats with an average weight of 170.81 ± 5.25g were acclimatized for one week and completely randomized into 5 different groups of four animals each (A-E). The animals in group A (normal control) received 1ml of distilled water, while animals in group B-E received 1mg/kg b.wt of Letrozole daily for 21 days orally (Kafali *et al* ., 2004). Thereafter, 24hrs after the last dose of letrozole was given, group B (PCOS-induced) received 1 ml of distilled water, group C (PCOS-induced) received 0.5ml of 7.14 mg/kg body weight of metformin and 0.5ml of 2mg/kg body weight clomiphene citrate, group D (PCOS-induced) received 0.5ml of 50 mg/kg b.wt of ethanolic extract of *Parquetina nigrescens* leaves and group E (PCOS-induced) each received 0.5ml of 100 mg/kg b.wt ethanoic extract of *Parquetina nigrescens* leaves. The extract administration was orally done once daily for 14 days .

2.6 Preparation of Serum

Serum preparation was done as described by Yakubu *et al*. (2008). The rats were weighed individually and thereafter anaesthetized in a jar containing cotton wool soaked in diethyl ether. The neck area was cleared of fur and skin to expose the jugular veins. The jugular veins were displaced slightly from the neck region and thereafter cut with a sharp sterile blade. The animals were held head downwards, allowed to bleed into clean, dry centrifuge tubes and left at room temperature for 10 minutes to clot. The blood samples were centrifuged at 3000rpm for 10 minutes using Thermo Scientific Centrifuge (Heraeus Megafuge 8). The sera were thereafter aspirated using Microflux pipette into clean, dry, sample bottles and were then stored frozen (4ºC) overnight before being used for the various biochemical assays.

2.7 Preparation of liver

Liver preparation was done as described by Yakubu *et al*. (2008).

2.8 Determination of Biochemical Parameters

The activities of ALP, AST, and ALT and concentrations of uric acid, creatinine, albumin, globulin, total and conjugated bilirubin, urea was determined by using standard procedures as described in the manufacturer's leaflet.

2.9 Statistical Analysis

Data were expressed as the mean ± standard error mean of four determinations and data were analyzed for significant at $P<0.05$ using One Way Analysis of Variance and Duncan Multiple Range Test performed with Statistical Package for Social Sciences, version 18.0 (SPSS Inc., Chicago, USA).

3.0 Results

The safety evaluation tests carried out on letrozole-induced PCOS rats administered EEPNL (table 1 and 2) reveals that there was a significant increase ($P<0.05$) in the serum creatinine uric acid and total bilirubin level of the PCOS compared to the control, while there was no significant difference

(P≥0.05) in the urea, albumin, globulin and albumin-globulin ratio level of the PCOS compared to the control. The BUN-Creatinine ratio and direct bilirubin concentration of PCOS animals was significantly decreased (P<0.05) when compared to the control. All the doses of EEPNL administered significantly increased (P<0.05) the serum concentrations of urea, uric acid, BUN-Creatinine ratio, albumin and total protein levels while the extract significantly decreased (P<0.05) the creatinine level in a manner similar to the control animals (Table 1 and 2). Furthermore, the direct bilirubin level and the albumin-globulin ratio of PCOS rats administered 50 mg/kg b.wt. of EEPNL compared favourably (P≥0.05) with the control animals while 100 mg/kg b.wt. of the extract significantly (P<0.05) decreased the direct bilirubin level in a manner similar to the control animals.

There was a significant decrease (P<0.05) in the concentration of total protein in PCOS rats administered reference drug when compared to the control, while there was an increase in the other letrozole-induced treatment groups when compared to the control (Table 2)

The concentrations of urea, uric acid and BUN-creatinine ratio of PCOS rats were significantly increased (P<0.05) by the administration of the reference drug (clomiphene citrate co-administered with metformin) when compared to the control.

This trend of elevation was observed in the liver ALT and serum ALP activity of PCOS rats treated with distilled water (Table 3 and 4). In contrast, the extract caused a decrease (P < 0.05) in the activitities of liver AST, ALT and ALP in manner similar to the animals in the control groups (Table 3 and 4). Furthermore, the activities of serum AST, ALT and ALP were significantly increased (P>0.05) by the extract at all the doses when compared to the non-PCOS animals administered distilled water only (Table 3 and 4). The administration of the reference drug to PCOS animals significantly increased (P < 0.05) the activities of serum ALP and liver ALT when compared to the control while the serum activities of AST and ALT and liver ALP were significantly decreased (P < 0.05) when compared to the control group (Table 3 and 4).

Table 1: Effect of ethanolic extract of *P. nigrescens* on kidney function indices

	Creatinine (mg/dl)	**Urea (mg/dl)**	**BUN Creatinine Ratio (mg/dl)**	**Uric acid (mg/dl)**
Control	0.34 ± 0.04 [a]	1.05 ± 0.26 [a]	2.98 ± 0.44 [a]	0.64 ± 0.00 [a]
PCOS + distilled water	0.54 ± 0.00 [b]	0.89 ± 0.00 [a]	1.68 ± 0.00 [b]	2.27 ± 0.00 [b]
PCOS+ Met + CC	0.50 ± 0.00 [b]	3.57 ± 0.00 [b]	6.54 ± 0.00 [c]	3.34± 0.00 [b]
PCOS + 50mg/kg b.w of EEPNL	0.20 ± 0.01 [c]	1.64 ± 0.09 [b]	8.18 ± 0.20 [c]	2.12 ± 0.53 [b]
PCOS + 100mg/kg b.w of EEPNL	0.10 ± 0.00 [c]	1.49 ± 0.09 [b]	14.90 ± 0.87c	7.33 ± 0.00 [b]

Data are means of four determination ± SEM. Values with different superscript are significantly different (P<0.05).

Table 2: Effect of ethanolic extract of *P. nigrescens* leaves on the concentration of the liver function indices.

Groups	Albumin (mg/dL)	Globulin (mg/dL)	Total Protein (mg/dL)	Direct Bilirubin (mg/dL)	Total Bilirubin (mg/dL)	Albumin -Globulin ratio (mg/dL)
Control	6.81 ± 0.69^{a}	5.60 ± 1.01^{a}	1.22 ± 0.33^{a}	5.01 ± 0.82^{a}	1.56 ± 0.55^{a}	1.28 ± 0.11^{a}
PCOS + distilled water	6.84 ± 0.08^{a}	5.07 ± 0.15^{a}	1.77 ± 0.23^{b}	1.29 ± 0.68^{b}	2.28 ± 0.28^{b}	1.36 ± 0.05^{a}
PCOS + Met + CC	7.12 ± 0.12^{a}	6.97 ± 0.11^{a}	0.16 ± 0.20^{a}	8.56 ± 0.52^{c}	0.31 ± 0.16^{c}	1.02 ± 0.00^{b}
PCOS + 50mg/kg b.w of EEPNL	9.32 ± 1.19^{b}	7.02 ± 2.16^{a}	2.31 ± 1.11^{b}	5.53 ± 2.29^{a}	0.39 ± 0.18^{c}	0.90 ± 0.29^{a}
PCOS + 100mg/kg b.w of EEPNL	8.04 ± 0.67^{b}	5.64 ± 1.86^{a}	4.48 ± 1.74^{b}	2.80 ± 0.65^{b}	5.66 ± 4.44^{d}	3.24 ± 1.97^{c}

Data are means of four determinations ± SEM. Values with different superscript are significally different (P<0.05).

Table 3: Effects of ethanolic extract of *P. nigrescens* leaves on the aminotransferase activity in the liver and serum of Letrozole-induced PCOS in female rats

Groups	Serum AST	Liver AST	Serum ALT	Liver ALT
Control	69.08 ± 0.05^{a}	106.08 ± 0.05^{a}	21.00 ± 0.00^{a}	75.00 ± 0.00^{a}
PCOS+ distil. H_2O	62.08 ± 0.05^{b}	94.08 ± 0.05^{b}	22.00 ± 0.00^{b}	108.00 ± 0.00^{b}
PCOS + Met +CC	28.08 ± 0.05^{c}	16.08 ± 0.05^{c}	13.00 ± 0.00^{c}	77.00 ± 0.00^{c}
PCOS + 50mg/kg b.w. of EEPNL	104.23 ± 0.13^{d}	104.08 ± 0.05^{d}	32.55 ± 0.06^{d}	75.65 ± 0.07^{d}
PCOS + 100mg/kg b.w. of EEPNL	87.58 ± 0.10^{d}	47.08 ± 0.05^{e}	54.73 ± 0.08^{e}	67.63 ± 0.10^{e}

Data are means of four determinations ± SEM. Values with different superscripts for each group are significantly different (P<0.05). MET-Metformin, CC- Clomiphene citrate, AST- Aspartate aminotransferase, ALT- Alanine aminotransferase.

Table 4: Effects of ethanolic extract of *P. nigrescens* leaves on alkaline phosphatase activities in the liver and serum of letrozole-induced PCOS in female rats

Groups	Serum ALP	Liver ALP
Control	11.88 ± 0.00^{a}	889.32 ± 0.00^{a}
PCOS+ distil. H_2O	297.05 ± 0.00^{c}	361.03 ± 0.00^{e}
PCOS + Met +CC	216.62 ± 0.00^{b}	95.06 ± 0.00^{c}
PCOS + 50mg/kg b.wt of EEPNL	836.43 ± 0.10^{e}	112.40 ± 0.03^{d}
PCOS + 100mg/kg b.wt of EEPNL	512.62 ± 0.13^{d}	87.32 ± 0.11^{b}

Data are means of four determinations ± SEM. Values with different superscripts for each group are significantly different (P<0.05). MET-Metformin, CC- Clomiphene citrate, ALP- Alkaline phosphatase

4.0 Discussion

The evaluation of the ethanolic extract of *P. nigrescens* on letrozole induced polycystic ovarian syndrome in female albino rats has given an insight to safety profile of the leave in the normal function of the blood, kidney and liver of the animal.

The kidney is known for its ability to eliminate metabolic waste, maintain balance at optimum pH and maintain chemical balance in the blood (Gounden *et al.,* 2021). It has been reported that

creatinine, urea and uric acid are considered to be metabolic waste thus high level of this in blood indicates kidney dysfunctions, damage or diseases (Gounden *et al.*, 2021). The decrease in serum creatinine levels of PCOS rats treated with the extract of EEPNL indicate inadequacy in the process of filtration at the glomerulus. Furthermore, the increased concentration of urea and uric acid in PCOS rats administered 50 and 100 mg/kg b.wt.of EEPNL suggests that these doses affect the kidney filtration of these waste. Also, increase in serum uric acid content of the animals could also imply reduced excretion of the chemical compound by the kidney (Yakubu and Nurudeen, 2014). High concentration of uric acid can lead to formation of crystals in a joint which may progress into kidney stone and eventually damage the kidney. All these alterations corroborate kidney dysfunction resulting probably from interference with the elimination and processing of the metabolite, inefficient filtration by the kidney and obstruction of the lower urinary tract, impaired glomerular and tubular reabsorption, or excretion of these ions (Yakubu *et al*., 2012). The serum BUN: Creatinine ratio measures the amount of nitrogen in the blood, and can also be used to distinguish whether the liver or kidney is affected since urea is produced by the liver and excreted by the kidney (Yakubu *et al*., 2012). Therefore, an increase in the computed BUN: Creatinine suggests that the elevated creatinine in the serum of the animals might be as a result of kidney dysfunction.

The liver function indices such as the serum globulin, albumin, and bilirubin concentration can serve as important factors in determining the secretory ability and the state of the liver. Changes in these concentrations of these parameters may denote the extent of damage done and the type of liver damage (Yakubu *et al*., 2012). The administration of the extract at 100mg/kg showed an increase level of the serum concentration of these metabolites because, letrozole causes an increase in the level of the concentration of albumin, globulin and bilirubin hence, showing that this dose might be probable cause of damage to the functionality of the liver. Moreover, the evaluation of albumin, globulin, and bilirubin (total and conjugated) in the serum of animals following the administration of chemical compounds including this plant extract cannot be overemphasized as they are useful criteria for assessing not only the secretory ability and or functional capacity of the liver but also the types of liver damage (Yakubu *et al.*, 2003). The lack of significance effect of the 50 mg/kg b.wt of EEPNL on PCOS rats in the level of globulin, direct bilirubin and albumin-globulin ratio might suggest that the extract at this dose may not induce any adverse effect on the normal functioning of the liver of the animals.

The increase in the activity of enzymes in the serum, could be attributed to either inhibition of the enzyme activity at the cellular/molecular level (Akanji *et al*., 1993), or inactivation of the enzyme molecules in situ (Umezawa and Hooper, 1982). AST is found mostly in the liver, but also in muscles. If liver damage occurs, it releases AST into the bloodstream. Doses at both 50 and 100mg/kg showed a reduced level in the concentration reversing the effect of letrozole which causes a spike in the level of AST concentration showing that both doses poses no damage and even reverses any damage caused. ALP can also reveals the functionality of the plasma membrane and a significant increase in the level of this enzyme denotes compromise in the integrity of the

plasma membrane. Administration of letrozole shows a diminished level of ALP activities and after administration of extract there was a significant decrease in the level of the ALP activity in the liver and serum showing that the extract inhibits the action of this enzyme similar to that of letrozole. ALT enzyme denotes the metabolic conditions of the hepatocytes and a high level of this enzyme shows damages done to the hepatocytes. ALT level was unchanged after administration of letrozole showing no negative effect while there was an increase in the level of this enzyme after the administration of the extract at both doses.

5.0 Conclusion

The ethanolic extract of *P. nigrescens* leaves has mildly affected the normal functioning of the liver and kidney of the PCOS rats. Therefore, the extract might be safe at the doses investigated as a treatment option in PCOS rats. The mild alterations produced by the extract at the doses investigated, when compared with the clomiphene citrate and metformin, may be subjected to further studies in order to establish and corroborated this claim.

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