



POLYPHENOL TREATMENT - A PANACEA FOR BENIGN PROSTATIC HYPERPLASIA-TRIGGERED IMMUNE INSULTS

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ABSTRACT

Benign prostatic hyperplasia (BPH) is the most common male benign proliferative disease; characterized by overgrowth of prostatic tissue around the urethra, constricting the urethral opening. This study investigated the effect of the polyphenols, *Kolaviron* and *Quercetin* on haematological parameters of testosterone-induced benign prostatic hyperplasia in Wistar rats. Fifty (50) animals ranging in weight between 250 and 300g were divided into 6 groups of 7 animals each. Group 1 served as normal control and received 0.5ml Canola oil, groups 2 to 6 were induced BPH via intraperitoneal injection of 5mg/kg body weight testosterone for 4 weeks. BPH was thereafter confirmed by sacrifice of three animals and collection of blood samples for PSA assay. Following successful induction of BPH, groups 2 received 0.5ml Canola oil while 3, 4, 5 and 6 were treated with 5mg/70kg body weight Finasteride (standard control), 150mg/kg body weight Kolaviron, 15mg/kg body weight Quercetin, and 150mg/kg body weight Kolaviron + 15mg/kg body weight Quercetin respectively. All treatments lasted for 28 days, after which animals were sacrificed and whole blood collected into anticoagulant-coated sample bottles, and used for haematological analysis. Testosterone administration resulted in reduction of red blood cells (RBC), haemoglobin (HB), and haematocrit (HCT). Treatments with both kolaviron and quercetin elevated the levels of RBC, HB and HCT. Furthermore, testosterone administration caused increases in white blood cells (WBC), lymphocytes (LYM), granulocytes (GRA) and other white blood cells (MID). All of the elevated white blood cell levels were reversed by treatment with kolaviron and quercetin, both and individual polyphenols and as a combined therapy. There were also reductions in mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) following induction of BPH. And these reductions were reversed by the treatments with both polyphenols, kolaviron and quercetin. Conclusively, the results suggest polyphenols such as Kolaviron and Quercetin might enhance immune response and might be a panacea to anaemic conditions arising from BPH.

Keywords: Testosterone, Benign Prostatic Hyperplasia, Kolaviron, quercetin, and Full Blood Count.

1.0 INTRODUCTION

Benign prostatic hyperplasia (BPH) is an age-dependent ailment that is very common in men, and affects the prostate gland. It is predisposed as an enlargement of the prostate

gland, which can lead to symptoms such as increased frequency of urination, difficulty starting and stopping urination, weak urinary stream, and the sensation of incomplete bladder emptying; altogether referred to as

lower urinary tract symptoms (LUTS). At the moment, the exact cause of BPH is not clearly understood, and its poorly deciphered mechanism of pathogenesis has been reported to be multifactorial. It is believed in some quarters that, hormonal imbalances, particularly an increase in testosterone and its metabolite, dihydrotestosterone (DHT), play a significant role in the development and progression of the condition (Kumar *et al.*, 2021).

The age-related decline in testosterone levels in the plasma, as well as an age-dependent decrease in the testosterone/estrogen ratio, with a resultant elevation in estrogen activity arising from the cyclization of testosterone by aromatase to produce estrogen. Hence, high estrogen activity may facilitate the hyperplasia of prostate cells (Suzuki, 1995; Radlmaier, 1996; Paglone, 2010). Another theory postulates that in older men, there is an increase in the activity of 5α -reductase, an enzyme which converts testosterone to DHT, thereby leading to a decrease in the testosterone/DHT ratio. The high levels of DHT may promote the growth of prostate cells, giving rise to hyperplasia (Ejike & Ezeanyika, 2008).

While the exact etiology of BPH is not fully understood, it is believed to be driven by hormonal imbalances, particularly an increase in the ratio of estrogen to testosterone. This increase in estrogen levels, combined with the corresponding decrease in testosterone levels, is believed to drive the proliferation of prostate cells and the development of BPH (Kumar *et al.*, 2021). BPH is histologically defined by a proliferation of the epithelial and stromal components within the prostate tissue. It arises in the urethra and transition zones of the prostate gland (Lepor, 2005). Current scientific proof suggests that prostate glandular carcinoma is a lot common in patients with BPH than those without it (Orsted & Bojesen, 2012).

Currently, the most common treatments for BPH are drugs that target the production or activity of DHT, such as finasteride and dutasteride. However, these drugs can have side effects such as sexual dysfunction, and their long-term safety is not fully understood (Roehrborn, 2008). Polyphenols, such as kolaviron and quercetin, have been proposed as alternative treatments for BPH. Kolaviron is a biflavonoid complex from *Garcinia kola*, while quercetin is a flavonoid found in many fruits and vegetables. Both compounds have been shown to have anti-inflammatory and anti-proliferative effects on prostate cells, making them potential candidates for the treatment of BPH (Gbadamosi *et al.*, 2021). Recent studies have shown promising results for the use of kolaviron and quercetin in the treatment of BPH. In a randomized controlled trial, supplementation with quercetin was found to improve LUTS and quality of life in men with BPH. Similarly, a study in rats found that kolaviron supplementation reduced prostate size and improved LUTS (Kalu *et al.*, 2016; Akinola *et al.*, 2020).

Haematological indices are a group of numerical values that describe the characteristics of blood cells in the circulation. These indices are obtained from a Full Blood Count (FBC) test, which is a common laboratory test used to evaluate a patient's overall health status. It provides information about the number and types of blood cells present in a person's bloodstream. A FBC typically includes measurements of RBC, WBC, and platelets count, as well as various other haematological parameters like MCV, MCH, MCHC, HB, HCT, and Red Cell Distribution Width (RDW). It is a useful test for diagnosing a variety of conditions, including anemia, infection, inflammation, leukemia and other conditions that affect the production or function of blood cells (Lippi *et al.*, 2015; Erhabor *et al.*, 2021; Dasofunjo *et al.*, 2023).

2.0 MATERIALS AND METHODS

Testosterone propionate manufactured by Greenfield Pharma, Jiangsu, China, was

bought from ND-Harris & associates Onitsha, Nigeria. Quercetin ($\geq 95\%$ HPLC) was acquired from Sigma-Aldrich Co. (St. Louis, MO, USA) through ND-Harris & associates Onitsha, Nigeria. Whereas Finasteride was purchased from Fidelity Medical Store, Igoli-Ogoja, Cross River State, Nigeria.

Collection of *Garcinia kola* and extraction of Kolaviron: *Garcinia kola* seeds were purchased from a local seller in Boki, Cross River State, Nigeria. Kolaviron was extracted from the healthy seeds of *G. Kola* and characterised in line with the method of Iwu (1993). Briefly, the pulverized seeds were extracted with petroleum ether (bp 40–60°C) using a Soxhlet for twenty-four hours. The defatted dried marc was repacked and extracted with acetone. The extract was concentrated and diluted double its volume with water and extracted with ethylacetate (6 × 300 ml). The concentrated ethylacetate fraction yielded a golden yellow solid called kolaviron; that was identified by direct comparison of the ^1H nuclear resonance (NMR), ^{13}C NMR, and negatron ionization (EI)-mass spectral results with antecedently revealed knowledge (Iwu, 1993).

Experimental animals: Fifty (50) Wistar rats ranging in weight between 250 and 300g were obtained from the Animal House of the Faculty of Basic Medical Sciences, University of Cross River State, Okuku campus, Nigeria. Following acclimatization to handling and experimental environment, they were housed in standard plastic cages

(60cm by 40cm dimension) provided with top mesh wire covers, under relative humidity (45%) and room temperature (26°C), with a 12-hour light-dark cycle. The rats were fed with pelletized rat chow and allowed access to water ad lib throughout the experimental period.

Induction and confirmation of BPH: Out of the fifty rats, forty were induced with BPH, and ten weren't. Testosterone propionate was used for the induction of BPH. A 5mg/kg body weight intraperitoneal injection of testosterone propionate was administered within the inguinal region of the forty experimental rats daily for four (4) weeks (Gasco et al., 2007). Once induction was completed, at the end of the fourth week, three (3) rats from the testosterone-treated and three from the non-treated cluster were arbitrarily chosen and sacrificed and examined for gross prostate enlargement (both gross and microscopic anatomical review of the prostate was doled out alongside the PSA concentration to indicate successful positive BPH induction).

Experimental design, animal grouping, and treatment

Thirty-five (35) rats from the pool of forty (40) rats induced with BPH were arbitrarily divided into five (5) sample groups (groups 2-6) consisting of seven (7) rats each, whereas seven rats (non-induced) constituted group one as shown in table 1 below. The rats were treated in line with the scheme in table 1.

Table 1. Experimental design, animal grouping and treatment

Group	Number of animals	Treatment
1	7	0.5ml Canola Oil
2	7	5mg/kg body weight testosterone + 0.5ml Canola Oil
3	7	5mg/kg body weight testosterone + 150mg/kg body weight kolaviron.
4	7	5mg/kg body weight testosterone + 15mg/kg body weight quercetin.
5	7	5mg/kg body weight testosterone + 150mg/kg body weight kolaviron + 15mg/kg body weight quercetin.
6	7	5mg/kg body weight testosterone +5mg/70kg body weight Finasteride. (control)

Sacrifice of experimental animals: After twenty-eight days of treatment with Canola oil, kolaviron and quercetin, as well as Finasteride, in line with the schedule in Table 1, the treatments were withdrawn twenty-four hours after the last dose administration and after a twelve hour fast, all animals were sacrificed under chloroform anaesthesia. Blood samples were collected via cardiac puncture for haematological analysis.

Haematological analysis: The full blood count and differential count were measured using auto haematology analyzer (Mindray Beston India).

Statistical analysis: Data obtained was analyzed by one-way ANOVA using Graph Pad Prism (version 8). All the values were expressed as Mean \pm Standard Error of Mean with the level of significance set at $p < 0.05$; $n=7$.

3.0 RESULTS

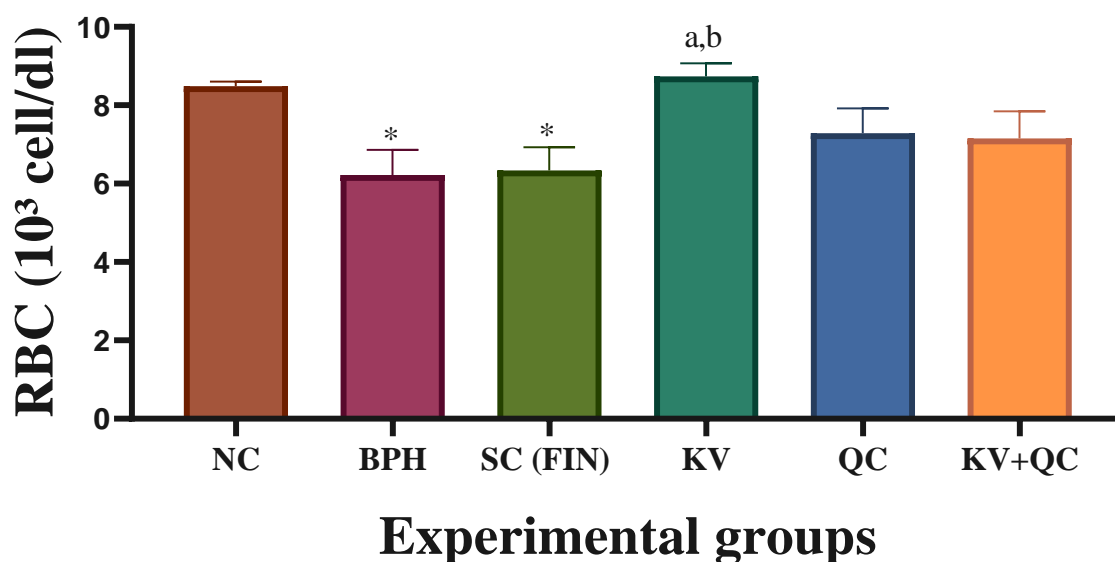


Figure 1: RBC concentration following BPH induction and treatment with Kolaviron and Quercetin in Wistar rats.

Values are expressed as Mean \pm SEM n=7.

*= significance difference compared with normal control, a= significance difference compared with BPH control, b= significance difference compared with standard control (finasteride) at = P<0.05.

NC=normal control, BPH= Benign prostatic hyperplasia, SC= standard control (finasteride), KV= Kolaviron, QC= Quercetin, KV+QC= Kolaviron+ Quercetin.

Figure 1 shows the effect of treatment on red blood cell. The result showed that BPH induction caused a significant decrease in RBC concentration shown by the decrease in BPH group vs NC group. Also, from the result it was observed that, the 28 days treatment caused a reverse in this effect in the treated groups with exception of the standard control (finasteride) treated group which further decreased after treatment. In the

treated groups a significant P<0.05 reduction in RBC concentration were recorded in standard control (finasteride) treated group vs NC, while Kolaviron treated group shows significant increase vs BPH and finasteride treated groups. Furthermore, non-significant P>0.05 effect of treatment were obtained in Kolaviron, Quercetin and Kolaviron + Quercetin treated groups vs NC group.

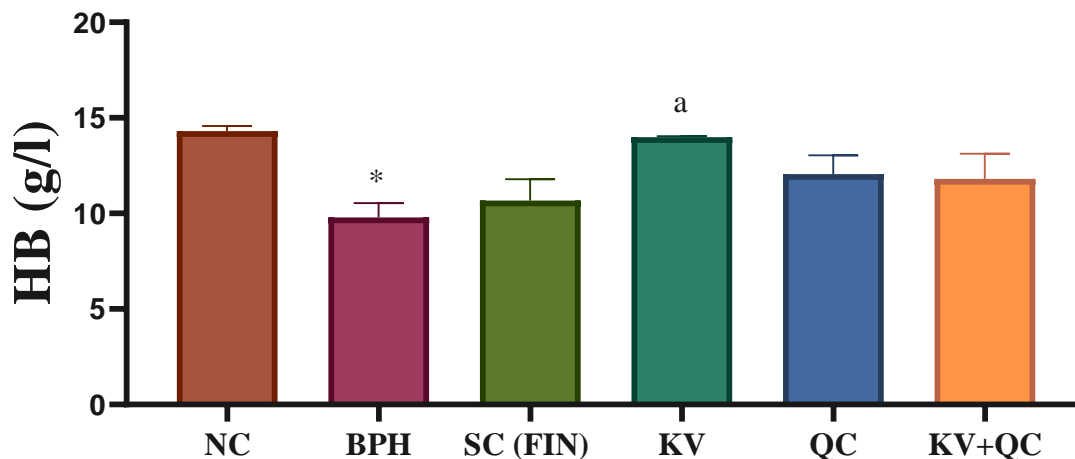


Figure 2: HB concentration following BPH induction and treatment with Kolaviron and Quercetin in Wistar rats.

Values are expressed as Mean \pm SEM n=7.

*= significance difference compared with normal control, a= significance difference compared with BPH group at = P<0.05

NC=normal control, BPH= Benign prostatic hyperplasia, SC= standard control (finasteride), KV= Kolaviron, QC= Quercetin, KV+QC= Kolaviron+ Quercetin.

Result obtained on HB level showed that BPH induction causes a significant P<0.05 decrease in HB level as obtained in BPH group vs NC group. Furthermore, the result showed that 28 days treatment caused a reverse in the level of HB significantly in all

treated groups Finasteride, Koaviron Quercetin and Kolaviron +Quercetin vs NC group. The result also showed significant P<0.05 increase in Hb level in Kolaviron treated group vs BPH group.

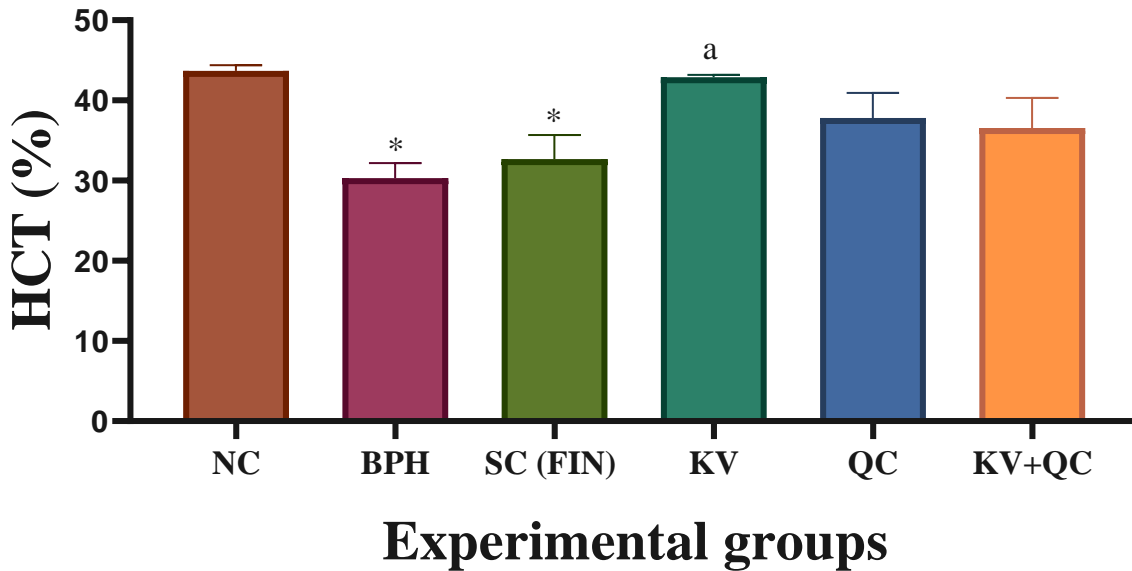


Figure 3: HCT concentration following BPH induction and treatment with Kolaviron and Quercetin in Wistar rats.

Values are expressed as Mean \pm SEM n=7.

*= significance difference compared with normal control, a= significance difference compared with BPH group at = P<0.05

NC=normal control, BPH= Benign prostatic hyperplasia, SC= standard control (finasteride), KV= Kolaviron, QC= Quercetin, KV+QC= Kolaviron+ Quercetin.

Figure 3 shows the effect of treatment on HCT. The result showed that BPH induction caused a significant decrease in HCT concentration shown by the decrease in BPH group vs NC group. Also, from the result it was observed that, the 28 days treatment caused a reverse in this effect in the treated groups with exception of the standard control (finasteride) treated group which further decreased after treatment. In the treated

groups a significant P<0.05 reduction in HCT concentration were recorded in standard control (finasteride) treated group vs NC, while Kolaviron treated group shows significant increase vs BPH group. Furthermore, non-significant P>0.05 effect of treatment were obtained in Kolaviron, Quercetin and Kolaviron + Quercetin treated groups vs NC group.

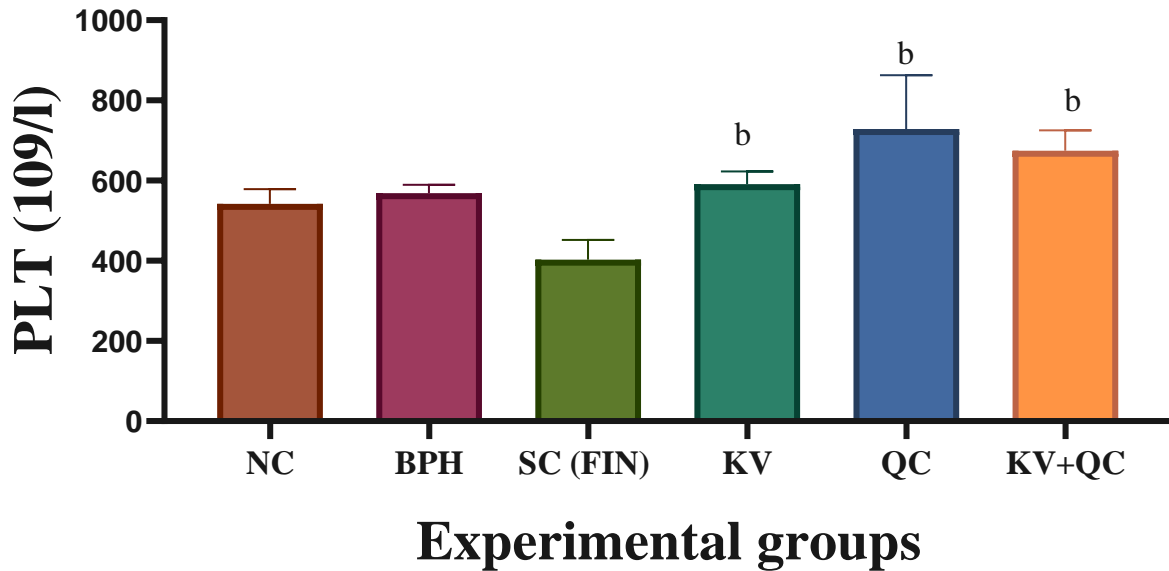


Figure 4: PLT concentration following BPH induction and treatment with Kolaviron and Quercetin in Wistar rats.

Values are expressed as Mean \pm SEM n=7.

b= significance difference compared with standard control (finasteride) at = P<0.05

NC=normal control, BPH= Benign prostatic hyperplasia, SC= standard control (finasteride), KV= Kolaviron, QC= Quercetin, KV+QC= Kolaviron+ Quercetin.

The was no significant P<0.05 effect in Platelet recorded in BPH induced group vs NC group and all treated groups Finasteride, Kolaviron, Quercetin and Kolaviron + Quercetin vs NC and BPH groups. The

results of this study also showed that the level of PLT were significantly P<0.05 increased in Kolaviron, Quercetin and Kolaviron + Quercetin treated groups vs standard control (finasteride).

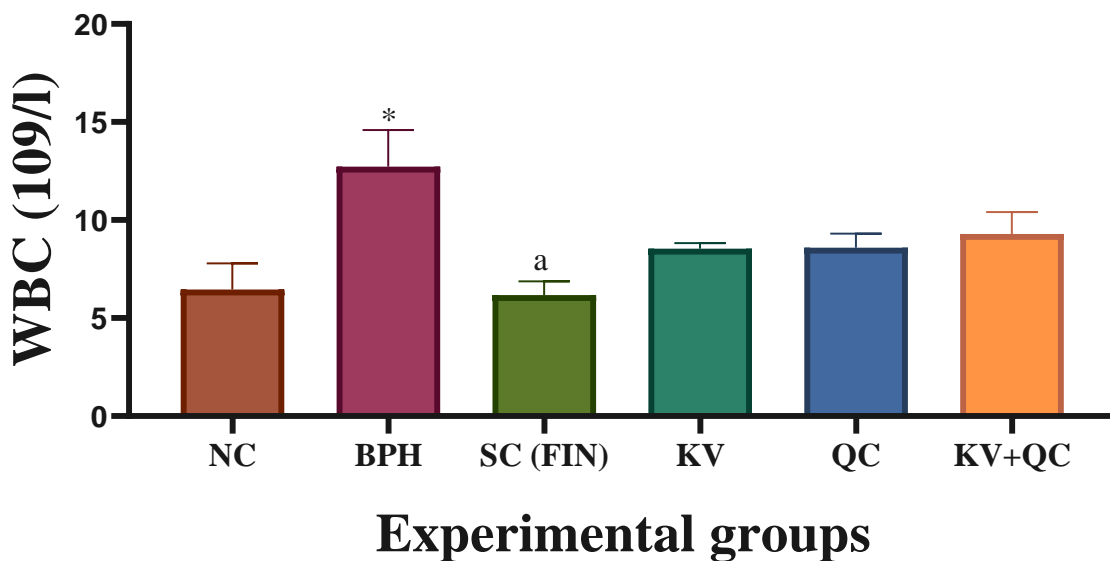


Figure 5: WBC concentration following BPH induction and treatment with Kolaviron and Quercetin in Wistar rats.

Values are expressed as Mean \pm SEM n=7.

*= significance difference compared with normal control, a= significance difference compared with BPH control (finasteride) at = P<0.05

NC=normal control, BPH= Benign prostatic hyperplasia, SC= standard control (finasteride), KV= Kolaviron, QC= Quercetin, KV+QC= Kolaviron+ Quercetin.

Results obtained on the concentration of white blood cell showed that BPH induction caused an increase in WBC concentration indicated in the increase obtained in BPH group vs NC group. Following 28 days treatment, the increase were reversed in the

treated groups significant P<0.05 in standard control (finasteride) vs BPH group. non-significant P>0.05 effect of treatment were obtained in finasteride, Kolaviron, Quercetin and Kolaviron + Quercetin treated groups vs NC group.

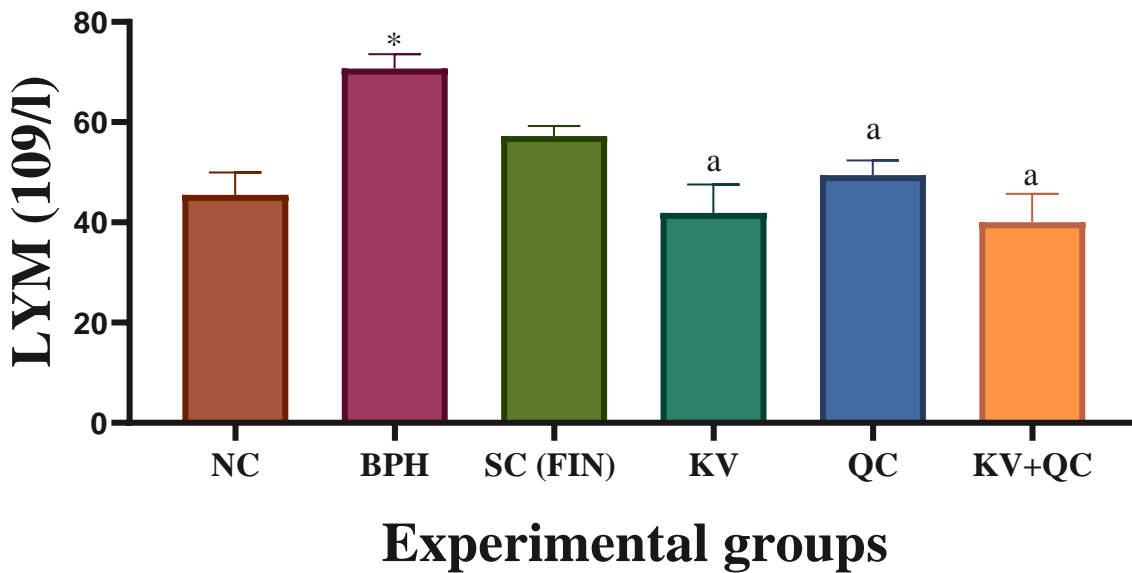


Figure 6: LYM concentration following BPH induction and treatment with Kolaviron and Quercetin in Wistar rats.

Values are expressed as Mean \pm SEM n=7.

*= significance difference compared with normal control, a= significance difference compared with BPH control, b= significance difference compared with standard control (finasteride) at = P<0.05

NC=normal control, BPH= Benign prostatic hyperplasia, SC= standard control (finasteride), KV= Kolaviron, QC= Quercetin, KV+QC= Kolaviron+ Quercetin.

Results obtained on the concentration of Lymphocytes showed that BPH induction caused an increase in LYM indicated in the increase obtained in BPH group vs NC group. Following 28 days treatment, the increase were reversed in the extract treated groups

significantly P<0.05 in, Kolaviron, Quercetin Kolaviron + Quercetin vs BPH group. non-significant P>0.05 effect of treatment were obtained in finasteride, Kolaviron, Quercetin and Kolaviron + Quercetin treated groups vs NC group.

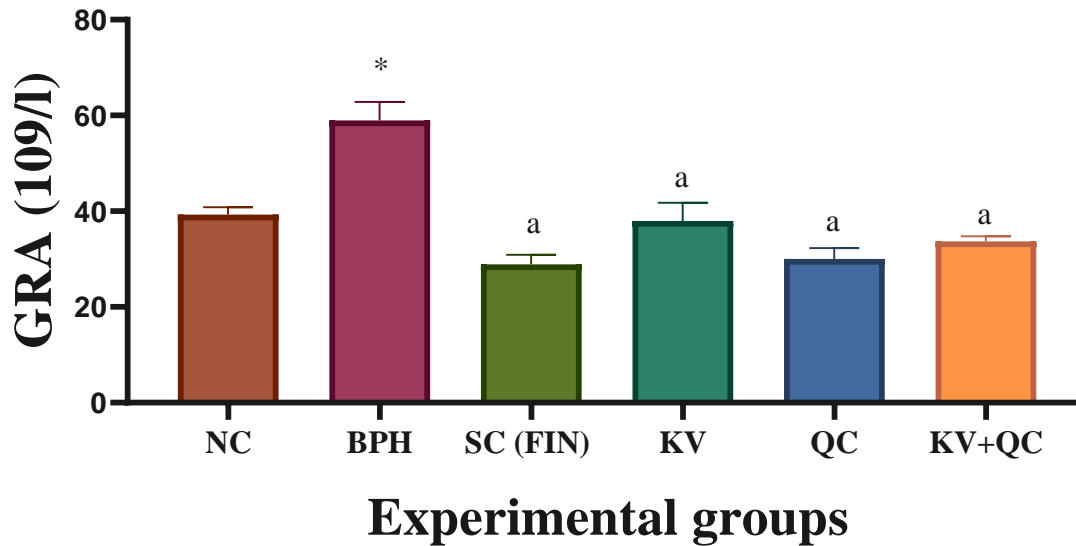


Figure 7: GRA concentration following BPH induction and treatment with Kolaviron and Quercetin in Wistar rats.

Values are expressed as Mean \pm SEM n=7.

*= significance difference compared with normal control, a= significance difference compared with BPH control, at = P<0.05

NC=normal control, BPH= Benign prostatic hyperplasia, SC= standard control (finasteride), KV= Kolaviron, QC= Quercetin, KV+QC= Kolaviron+ Quercetin.

Results obtained on the concentration of Granulocytes showed that BPH induction caused an increase in GRA indicated in the increase obtained in BPH group vs NC group. Following 28 days treatment, the increase were reversed in the extract treated groups

significantly P<0.05 in, Kolaviron, Quercetin Kolaviron + Quercetin vs BPH group. non-significant P>0.05 effect of treatment were obtained in finasteride, Kolaviron, Quercetin and Kolaviron + Quercetin treated groups vs NC group.

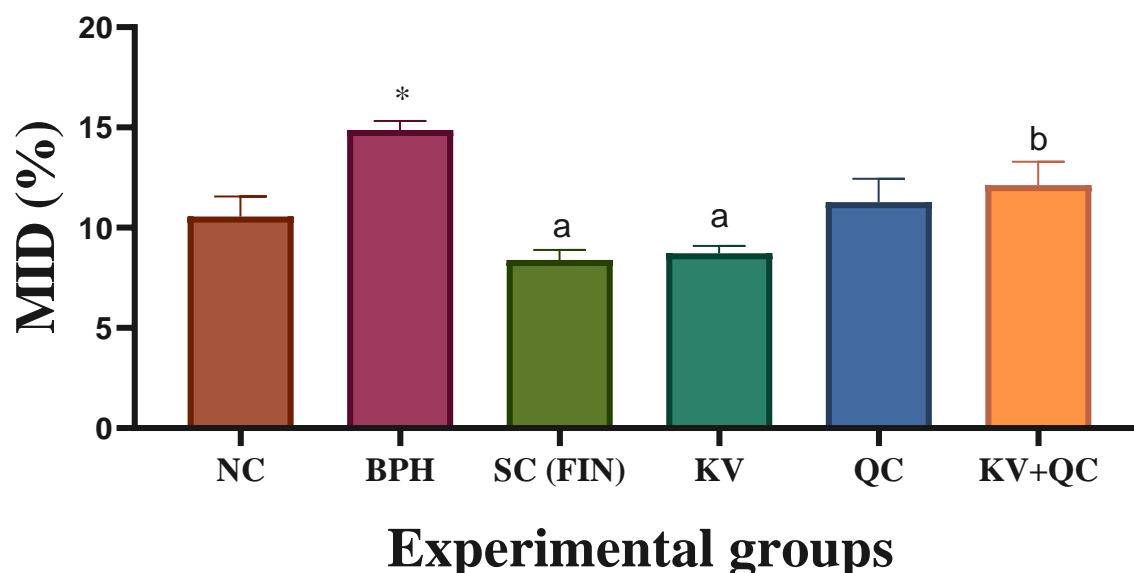


Figure 8: MID concentration following BPH induction and treatment with Kolaviron and Quercetin in Wistar rats.

Values are expressed as Mean \pm SEM n=7.

*= significance difference compared with normal control, a= significance difference compared with BPH control, b= significance difference compared with standard control (finasteride) at = P<0.05

NC=normal control, BPH= Benign prostatic hyperplasia, SC= standard control (finasteride), KV= Kolaviron, QC= Quercetin, KV+QC= Kolaviron+ Quercetin.

Results obtained on MID level showed that BPH induction caused an increase in MID indicated in the increase obtained in BPH group vs NC group. Following 28 days treatment, the increase were reversed in the extract treated groups significantly P<0.05 in standard control (finasteride) Kolaviron vs

BPH group. Quercetin, Kolaviron + Quercetin treated group showed no significant effect vs NC and BPH groups. Furthermore, a significant increase in MID was observed in Kolaviron + Quercetin treated groups vs standard control (finasteride) group.

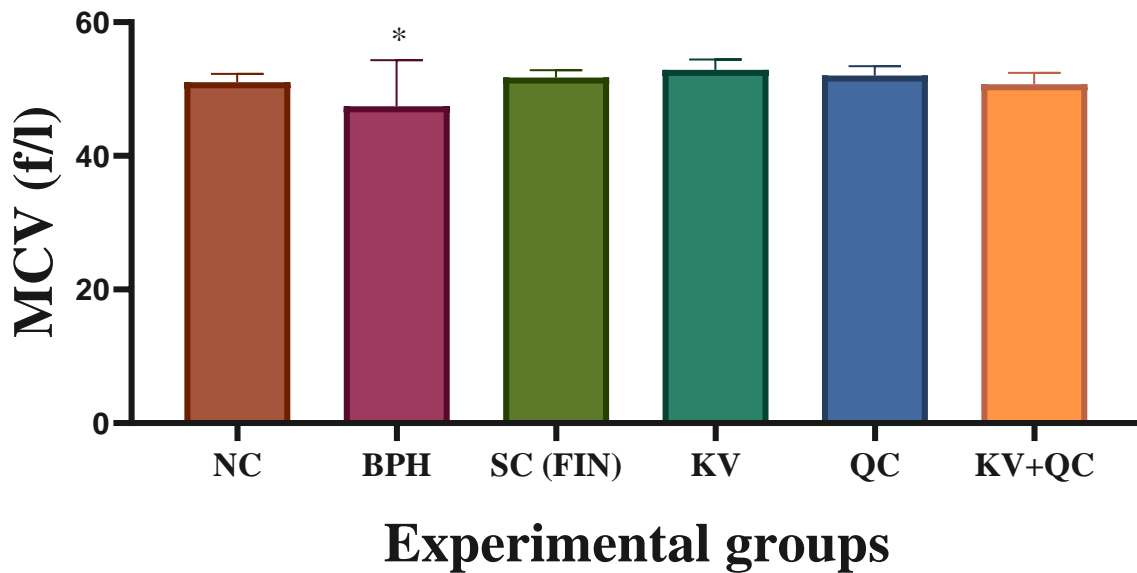


Figure 9: MCV following BPH induction and treatment with Kolaviron and Quercetin in Wistar rats.

Values are expressed as Mean \pm SEM n=7.

*= significance difference compared with normal control, a= significance difference compared with BPH control at = P<0.05

NC=normal control, BPH= Benign prostatic hyperplasia, SC= standard control (finasteride), KV= Kolaviron, QC= Quercetin, KV+QC= Kolaviron+ Quercetin.

Results obtained on MCV concentration showed that BPH induction caused an increase in MCV indicated in the increase obtained in BPH group vs NC group. Following 28 days treatment, the increase were reversed in the extract treated groups

significantly P<0.05 in standard control (finasteride) Kolaviron, Quercetin and Kolaviron + Quercetin vs NC group. Kolaviron + Quercetin treated group showed significant decrease in MCV concentration vs BPH group.

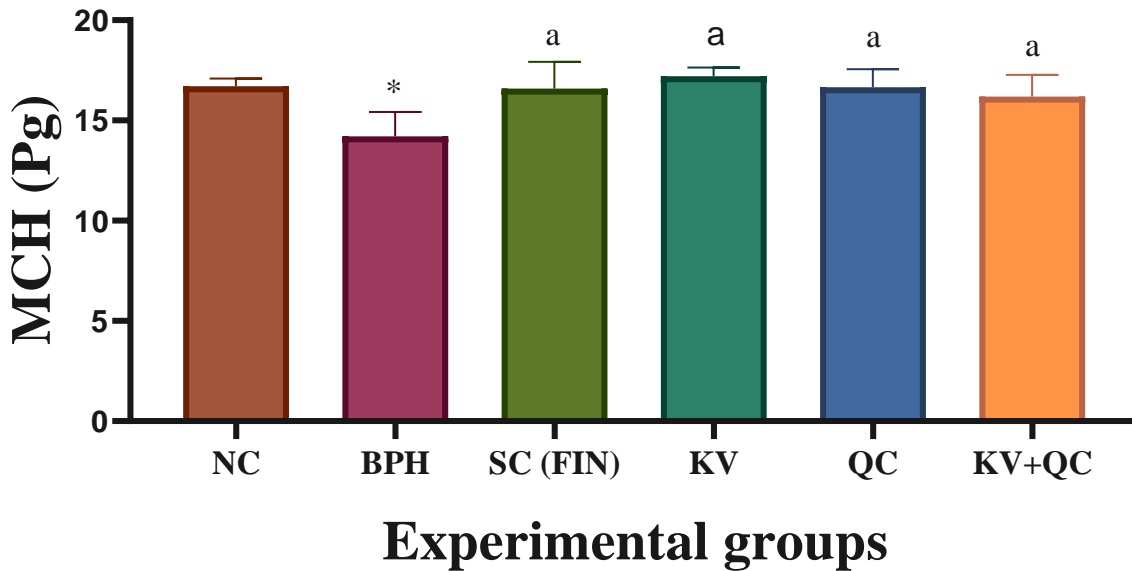


Figure 10: MCH concentration following BPH induction and treatment with Kolaviron and Quercetin in Wistar rats.

Values are expressed as Mean \pm SEM n=7.

*= significance difference compared with normal control, **a**= significance difference compared with BPH control at = P<0.05

NC=normal control, BPH= Benign prostatic hyperplasia, SC= standard control (finasteride), KV= Kolaviron, QC= Quercetin, KV+QC= Kolaviron+ Quercetin.

The result in figure 10 Showed reduction in MCH concentration in BPH group vs NC. After 28 days treatment, there was no significant effect observed in all treated groups, standard control (finasteride), kolaviron, Quercetin and Kolaviron +

Quercetin vs NC group. The result also showed significant increase in MCH concentration in standard control (finasteride), kolaviron, Quercetin and Kolaviron + Quercetin vs BPH group.

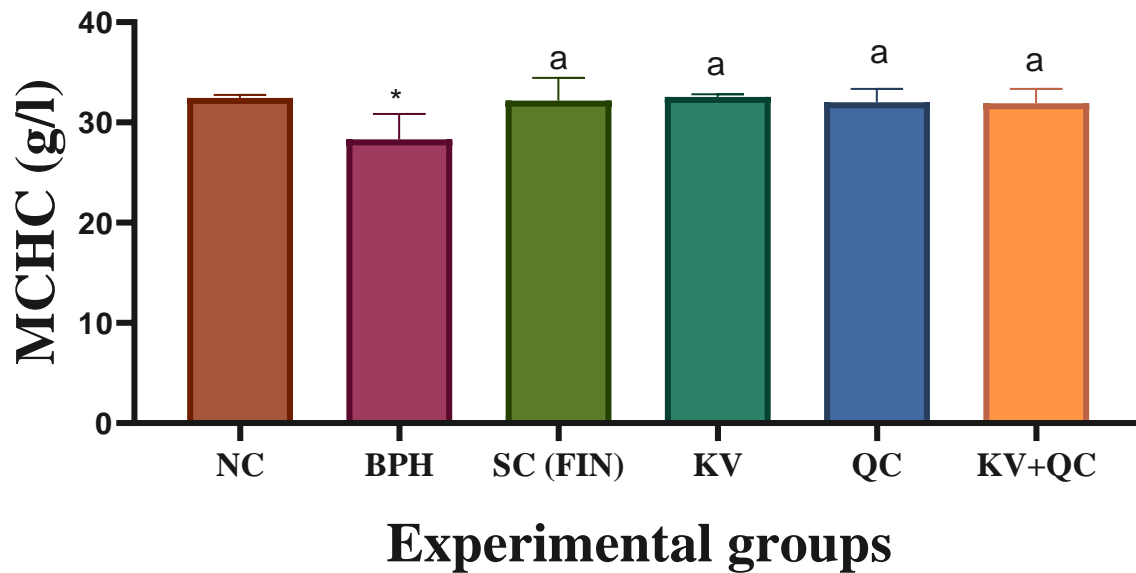


Figure 11: MCHC concentration following BPH induction and treatment with Kolaviron and Quercetin in Wistar rats.

Values are expressed as Mean \pm SEM n=7.

NC=normal control, BPH= Benign prostatic hyperplasia, SC= standard control (finasteride), KV= Kolaviron, QC= Quercetin, KV+QC= Kolaviron+ Quercetin.

The result as presented in figure 11 Showed reduction in MCHC concentration in BPH group vs NC. After 28 days treatment, there was no significant effect observed in all treated groups, standard control (finasteride), kolaviron, Quercetin and Kolaviron + Quercetin vs NC group. The result also showed significant increase in MCHC concentration in standard control (finasteride), kolaviron, Quercetin and Kolaviron + Quercetin vs BPH group.

4.0 DISCUSSION

The presence of bioactive ingredients found in Kolaviron and Quercetin has justified its use in traditional medicine for the treatment of various clinical conditions such as, inflammation, ulcer, diabetes etc. (Ayepola *et al.*, 2013; Xiao *et al.*, 2013). The haematological analysis was carried out to provide useful information on the general; state of the blood chemistry after administration of extracts following BPH induction. The results demonstrated BPH

induction in rats decreases the concentration of RBC, HB, and HCT.

Increased RBC and HB were observed in this present study following the administration of Kolaviron and Quercetin extracts. The observed increase in both RBC and HB could indicate erythrocytic synthesis (Dede *et al.*, 2002). Therefore, the observed increase in RBC count and HB may connote that the extracts enhances haematopoiesis and or erythropoiesis. Likewise, the oxygen transporting of the blood and the oxygen supplied to the tissues may be improved following the administration of the extract after BPH induction. Packed cell volume also known as haematocrit (HCT) or erythrocyte volume fraction (EVF) is the percentage (%) of red blood cells in blood. It measures the percentage volume of cells in the blood. Anaemic condition is associated with low production of red blood cell (Guenther & Lawrence, 2005). PCV is also involved in transportation of oxygen and absorbed nutrients. Increased PCV concentration may

depicts a better transportation and thus may result in an increased primary and secondary polycythaemia (Isaac *et al.*, 2013). The observed marked increase in PCV from this study suggest that *Kolaviron* and *Quercetin* may positively interfere with osmoregulatory and haematopoietic system of the blood that can enhance anaemia management (Audu *et al.*, 2014). It also suggests that the BPH induction may have altered the PCV chemistry.

This study further reveals normal levels of PLT in BPH induced group and the treatment groups relative to control. According to (McLellan *et al.*, 2003), normal PLT level in experimental rat indicate good action on the blood oxygen transporting ability as well as thrombopoietin. The observed normal PLT in this study therefore indicates that *Kolaviron* and *Quercetin* may improve the blood oxygen transporting ability following BPH induction. It also suggests that BPH induction may not alter the PLT level.

The results of this study further showed that BPH induction raises WBC, LYM, GRA and MID relative to control. After 28 days treatment with *Kolaviron* and *Quercetin* WBC, LYM, GRA and MID concentration were normal relative to control. This may suggest that the extract may help against invading pathogens possibly by phagocytosis or by generating antibodies that might enhance immune response possibly by immune modulatory or suppressant or adjuvant action. The major role of the white blood cells and its differential are to fight against infection, defend the body by phagocytosis from invading pathogens and to transport and distribute antibodies by immune response. Thus, animals with low white blood cells and white blood cells differentials are exposed to high risk of disease infection, while those with normal WBC and WBC differential counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to disease (Soetan *et al.*, 2013)

and enhance adaptability to local environmental and disease prevalent conditions (Nse-Abasi *et al.*, 2014). High levels of WBC and WBC differential count as observed in BPH group may suggest an infection, bone marrow disease, inflammation etc.

The mean corpuscular volume (MCV) is a measure of the average volume of red blood cell. In anemic condition, the MCV measurements allows for classification as either a microcytic anemia, normocytic anemia or macrocytic anemia (Tonnesen *et al.*, 2019). MCH stands for Mean corpuscular Hemoglobin, and is a calculation of the average amount of haemoglobin contained in each of a person's red blood cells (Tonnesen *et al.*, 2019). Mean corpuscular haemoglobin concentration (MCHC) is a measurement of the average amount of haemoglobin in a single red blood cell (RBC) as it relates to the volume of the cell (Tonnesen *et al.*, 2019). The results as observed in this study showed that BPH induced group cause a significant reduction in MCV, MCH and MCHC relative to control. This may depict anaemic condition induce by BPH. While extract treated groups showed significant increase in MCV, MCH and MCHC relative to BPH group. This may suggest that *Kolaviron* and *Quercetin* may positively interfere with osmoregulatory and haematopoietic system of the blood that can enhance anaemia management (Audu *et al.*, 2014).

5.0 CONCLUSION

This present study suggests that treatment with *Kolaviron* and *Quercetin* following BPH induction might fight against foreign infection possibly by phagocytosis or by generating antibodies that might enhance immune response. Furthermore, the extract might be a panacea to anaemic condition following BPH induction.

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