



COMBINED AMELIORATIVE ACTION OF *KOLAVIRON* AND *QUERCETIN* ON TESTOSTERONE PROPIONATE-INDUCED HEPATOTOXICITY OF BENIGN PROSTATIC HYPERPLASIA IN WISTAR RATS

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ABSTRACT

This research examined the impact of kolaviron and quercetin on hepatic toxicity in Wistar rats with testosterone propionate-induced benign prostatic hyperplasia. Forty-two animals weighing between 250 and 300g were utilized, divided into six groups, each comprising seven animals. Group one served as the normal control and received 0.5ml Canola oil, while group two- six underwent BPH induction via an intraperitoneal injection of 5mg/kg body weight testosterone for four weeks. Following BPH induction, group two got 0.5mL of Canola oil, while groups three, four, five, and six were treated with 150mg/kg body weight Kolaviron, 15mg/kg body weight Quercetin, 150mg/kg body weight kolaviron plus 15mg/kg body weight quercetin and 5mg/70kg body weight finasteride for twenty-eight days. Following the experimental duration, the rats were euthanized, and liver samples were collected for biochemical analyses. BPH induction led to a decrease in liver weight in rats compared to the control group, whereas treatment with Kolaviron, Quercetin, and Kolaviron + Quercetin resulted in an increase in liver weight compared to the BPH group. Moreover, BPH induction elevated hepatic concentrations of ALT, AST, ALP, total protein, albumin, globulin, total bilirubin, and conjugated bilirubin in comparison to the normal control group. Conversely, treatment with KV, QC, and KV+QC decreased the concentrations of ALT, AST, ALP, total protein, albumin, globulin, total bilirubin, and conjugated bilirubin compared to the BPH group. In conclusion, our study demonstrated that quercetin and kolaviron significantly reduced the hepatotoxicity in rats induced by testosterone propionate and could be a cheap and non-invasive treatment alternative for hepatic toxicity brought on by benign prostatic hyperplasia in men.

Keywords: Hepatic toxicity, Testosterone propionate, Kolaviron, Quercetin, Benign Prostatic Hyperplasia.

1.0 INTRODUCTION

In the male reproductive system, the prostate serves as a vital accessory gland. Among elderly men, the two prevalent proliferative disorders are prostate cancer and benign prostatic hyperplasia (BPH: Delella & Felisbino, 2010). BPH, a condition associated with aging, entails the non-malignant enlargement and uncontrollable growth of the prostate (Ejike & Ezeanyika 2011). Complications of BPH include sepsis, renal failure, irreversible bladder damage, and, in severe cases, even mortality. Despite

extensive research, the precise etiopathological mechanisms underlying BPH remain elusive. Hormonal regulation is implicated in this process, involving alterations in the androgen-to-estrogen ratio (Shin et al., 2012). The enzyme 5-alpha-reductase converts testosterone to dihydrotestosterone (DHT), which plays a pivotal role in prostate growth. The aging process and increasing DHT levels contribute to prostate hyperplasia. Additionally, prostate inflammation influences the rate of BPH progression (Sarbishegi et al., 2016).

The necessity to screen for potential sources of more effective treatment with low or no side effects has arisen due to the adverse effects and high cost of present treatment alternatives. Locally, Polyphenols, such as kolaviron and quercetin, have been proposed as alternative treatments for BPH. Kolaviron is a biflavonoid complex from *Garcinia kola*, while Numerous fruits and vegetables contain the flavonoid quercetin (Adaramoye, & Lawal, 2015). Both compounds have been reported to possess 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 in haematological and anti-oxidant indices from the use of kolaviron and quercetin in the treatment of BPH (Ujong & Okori, 2023; Ujong, 2023). As a result, a scientific evaluation of the attenuating ability of Kolaviron and Quercetin on Testosterone propionate-induced BPH in male Wistar rats was conducted.

2.0 MATERIALS AND METHODS

Testosterone propionate brand name: Recostrone, manufactured by Greenfield Pharma, Jiangsu Co Limited, China, was procured 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 a product of Aurobindo Pharma-Milpharm Ltd. ATC code: G04CB01 was purchased from Fidelity Medical Store, Igoli-Ogoja, Cross River State, Nigeria.

2.1 Collection of *Garcinia kola* and extraction of Kolaviron

We obtained *Garcinia kola* seeds from a local vendor in Boki, Cross River State, Nigeria. Kolaviron was then extracted from the healthy seeds of *G. kola* and characterized using a method established in previous studies (Iwu et al., 1993).

2.2 Experimental animals

Ethical clearance for the treatment and management of experimental animals was granted by the Animal Ethical Committee of the Faculty of Basic Medical Science, University of Calabar, under approval number 114BCM262. Forty-two Wistar rats weighing between 250 and 300g were procured from the Animal House at the Okuku Campus of the University of Cross River State, Faculty of Basic Medical Sciences. Upon acclimatization to handling and the experimental environment, the rats were accommodated in standard plastic cages (measuring 60cm by 40cm) equipped with top mesh wire covers, maintained at a room temperature of 26°C and relative humidity of 45%. They were provided with Mazuri pelletized rat chow and had unrestricted access to water throughout the experimental duration.

2.3 Induction and confirmation of BPH

Thirty-five (35) rats were induced with BPH, and seven were not. 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 propionate-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 Prostate specific antigen (PSA) concentration to indicate successful positive BPH induction.

2.4 Experimental design and treatment

Thirty-five (35) rats induced with BPH were randomly allocated into five (5) experimental groups (groups 2-6), each comprising seven (7) rats, while group one (1) consisted of seven non-induced rats, as illustrated in Table

1. Treatment of the rats followed the protocol outlined in Table 1.

2.5 Termination of experiment and Collection of samples for analysis

The rats underwent an overnight fast, were anesthetized under chloroform and sacrificed upon the trial's conclusion. Liver samples were harvested for biochemical assays.

2.6 Homogenization of liver

The collected organs were rinsed in ice-cold 1.15% KCl solution, gently dried with filter paper, and weighed using an electronic balance. Subsequently, they were homogenized on ice in 4 ml phosphate buffer (homogenizing buffer) with a pH of 7.4, utilizing a laboratory mortar and pestle. The resulting homogenate was then subjected to centrifugation at 10,000g for fifteen minutes at 4°C using a refrigerated centrifuge to obtain the post-mitochondrial fraction. The supernatant was carefully collected and stored in a laboratory freezer at -20°C until required for biochemical analyses.

2.7 Biochemical Analysis

Levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were analyzed using a double-beam spectrophotometer employing kinetic

techniques kits from Randox (United Kingdom). Measurements of total protein, albumin, globulin, total bilirubin, and conjugated bilirubin were conducted using kits obtained from Randox Laboratories Limited, United Kingdom (UK), following the manufacturer's guidelines.

2.8 Statistical analysis

All data were expressed as mean \pm SEM. Statistical analysis was conducted using Analysis of Variance with GraphPad Prism statistics, version 8.0. Differences were considered statistically significant at $p < 0.05$.

3.0 RESULTS

The results of organ (liver) weight (fig. 1) show that BPH induction decreases liver tissue weight compared to normal control, while all treated groups (KV, QC, KV+QC) show increase in liver tissue weight compared to BPH group.

Furthermore, BPH induction increases hepatic total bilirubin, total protein, albumin, and conjugated bilirubin compared to normal control. These increases were reversed in all treated groups (KV, QC, KV+QC) compared to BPH group (fig. 2,3,4 and 5).

TABLE 1: Experimental design, animal grouping and treatment

Groups	Number of animals	Treatment
1	7	0.5ml of vehicle (Tween 80 + Canola Oil in the ratio 1:10)
2	7	5mg/kg body weight testosterone + 0.5ml of vehicle
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.

TABLE 2: Effect of Kolaviron and Quercetin treatment on hepatic function indices.

Group	Hepatic AST conc. (IU/L)	Hepatic ALT conc. (IU/L)	Hepatic ALP conc. (IU/L)
Normal control	66.86 ± 1.079	29.00 ± 1.648	117.0 ± 2.449
BPH control	90.43 ± 1.429*	41.29 ± 0.778*	172.1 ± 1.933*
BPH + Kolaviron	80.29 ± 0.606 ^a	33.29 ± 0.606 ^a	155.3 ± 1.848 ^a
BPH + Quercetin	79.57 ± 2.034 ^a	37.00 ± 1.113 ^a	152.1 ± 1.696 ^a
BPH + KV + QC	60.86 ± 0.553 ^a	38.29 ± 1.229	160.6 ± 1.378 ^a
BPH + Finasteride	59.86 ± 0.634 ^a	30.14 ± 0.829 ^a	130.6 ± 1.212 ^a

Values are expressed as mean ± SEM; n=7.

Significant differences from the control group are denoted by * (p<0.05)

Significant differences from the BPH group are denoted by ^a(p<0.05).

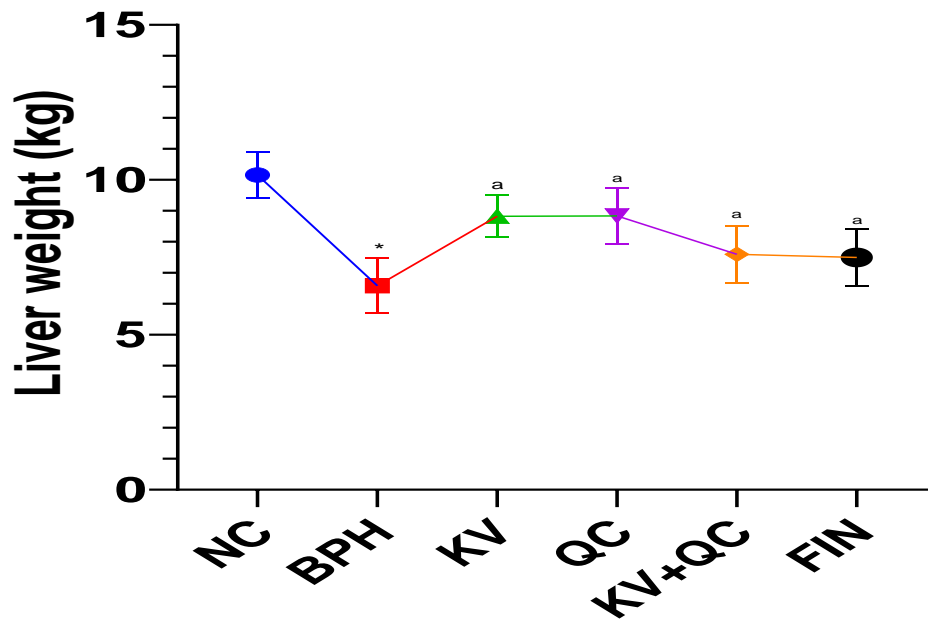


FIG.1: Effect of Kolaviron and quercetin on Liver weight

Values are given as mean ± SEM; n=7.

Significant differences from the control group are denoted by * (p<0.05)

Significant differences from the BPH group are denoted by ^a(p<0.05)

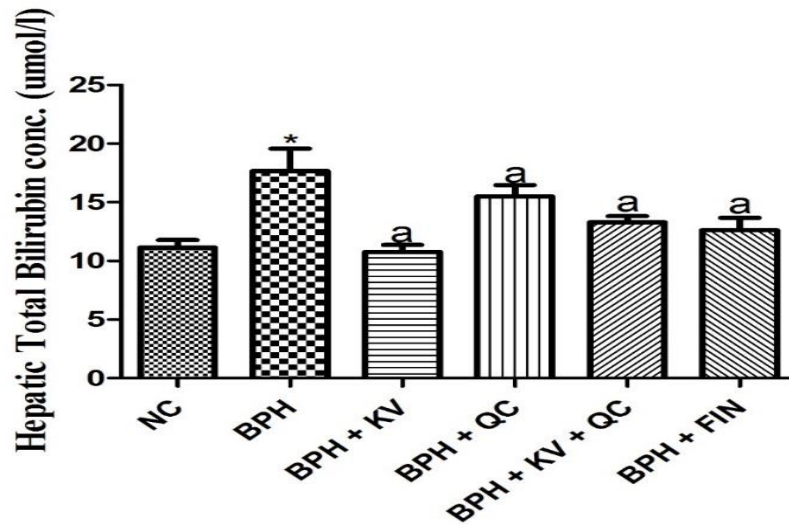


FIG.2

Significant differences from the control group are denoted by * ($p < 0.05$)
Significant differences from the BPH group are denoted by ^a ($p < 0.05$)

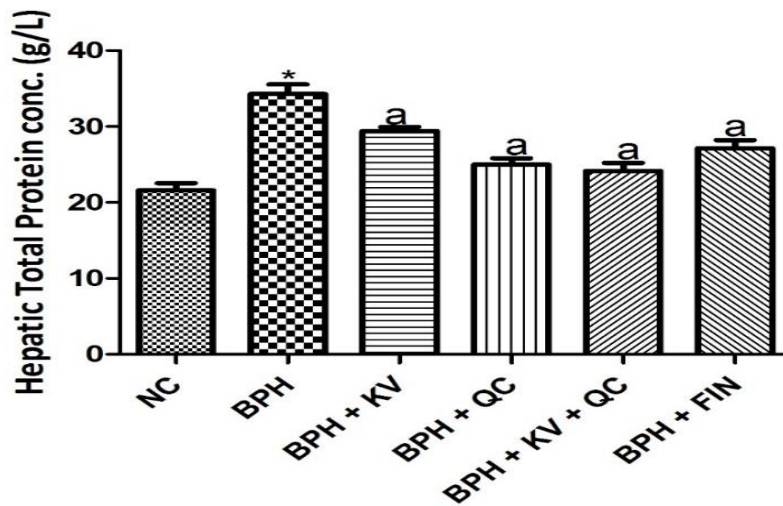
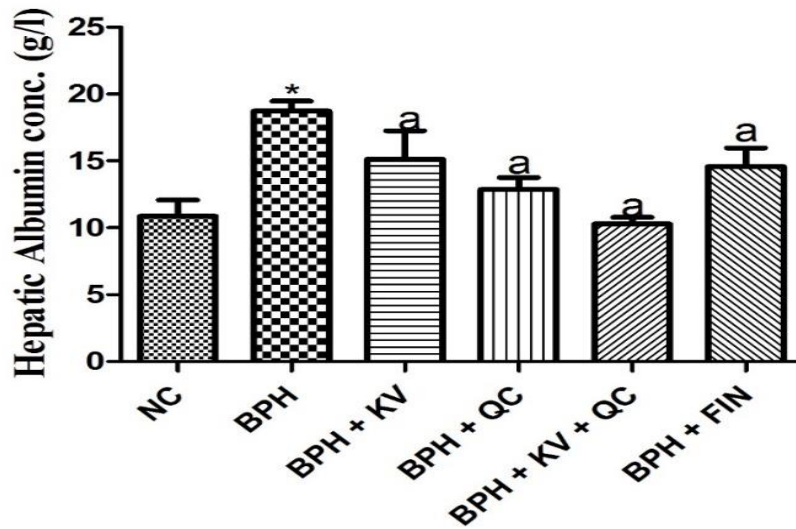


FIG.3: Effect of Kolaviron and quercetin on Hepatic Total Protein
Values are given as mean \pm SEM; n=7.

Significant differences from the control group are denoted by * ($p < 0.05$)
Significant differences from the BPH group are denoted by ^a ($p < 0.05$)



Significant differences from the control group are denoted by * ($p < 0.05$)
Significant differences from the BPH group are denoted by ^a ($p < 0.05$)

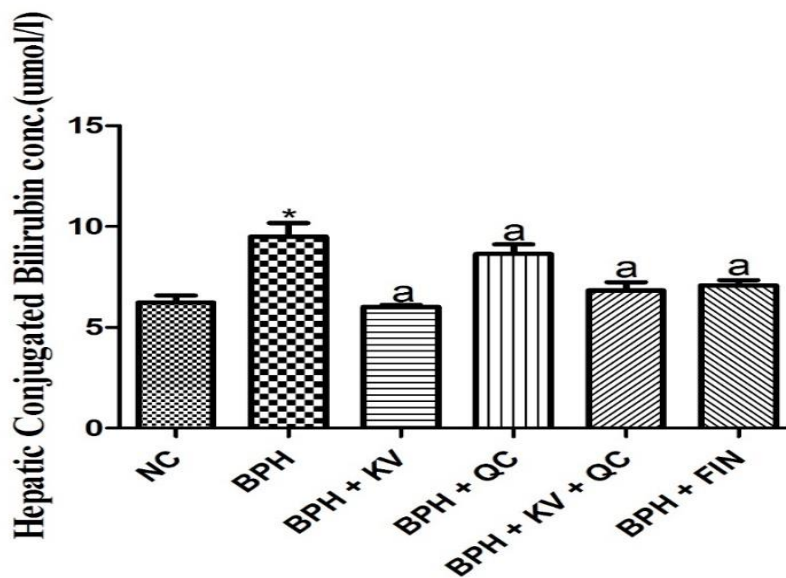


FIG.5: Effect of Kolaviron and quercetin on Hepatic Conjugated Bilirubin
Values are given as mean \pm SEM; $n=7$.
Significant differences from the control group are denoted by * ($p < 0.05$)
Significant differences from the BPH group are denoted by ^a ($p < 0.05$)

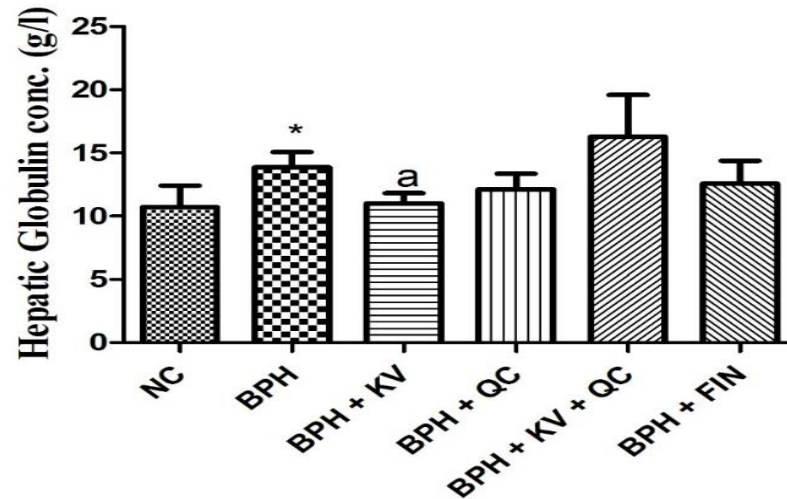


FIG.6: Effect of Kolaviron and quercetin on Hepatic Globulin

Values are given as mean \pm SEM; n=7.

Significant differences from the control group are denoted by * (p<0.05)

Significant differences from the BPH group are denoted by ^a (p<0.05)

The findings regarding hepatic globulin (Fig 6) exhibited elevated concentrations in the BPH group compared to the normal control. Treatment with KV alone resulted in a decrease in hepatic globulin concentrations compared to the BPH group. Conversely, both the group treated solely with QC and the group co-administered with KV and QC did not demonstrate any significant (p>0.05) variance in hepatic globulin concentrations compared to the BPH control group.

The hepatic levels of AST, ALT, and ALP (Table 2) exhibited elevated concentrations in the liver samples of the BPH group compared to the normal control. Conversely, treatment with KV, QC, and the combined administration of KV and QC resulted in notable reductions in AST and ALP concentrations. However, there were no significant differences (p>0.05) observed in ALT levels in the group co-treated with KV

and QC. Nonetheless, both the groups treated solely with KV and QC displayed significant decreases in hepatic ALT concentrations compared to the BPH group.

4.0 DISCUSSION

In this study, Testosterone propionate induction reduces liver weight of wistar rats. However, this pattern is overturned in all the treated groups involving KV, QC, and KV+QC, exhibiting a noticeable rise in liver tissue weight compared to the BPH group. These findings imply a potential safeguarding influence of Kolaviron and quercetin on liver tissue integrity amid BPH induction. The observed reduction in liver weight following induction of benign prostatic hyperplasia suggests a potential systemic impact of prostatic enlargement on hepatic morphology. While the precise mechanisms underlying this phenomenon remain unclear, several hypotheses warrant

consideration. One possibility is the alteration in hormonal milieu associated with BPH, including changes in androgen levels, which could influence hepatic metabolism and tissue composition. Additionally, systemic inflammation triggered by BPH may contribute to hepatic changes, given the liver's role in immune modulation and acute phase responses (Fibbi et al., 2010). Research by Liu *et al.* (2019), demonstrated that the induction of BPH in Wistar rats resulted in a significant decrease in liver weight compared to control groups. The study attributed this reduction to the systemic effects of androgen excess, which may influence hepatic metabolism and function. Moreover, alterations in hormonal balance induced by BPH induction could lead to changes in liver physiology, potentially affecting liver weight (Liu et al., 2019).

Specifically, these studies have also observed a decrease in hepatic total bilirubin, protein, albumin, conjugated bilirubin, and globulin levels, which could indicate hepatic dysfunction and altered protein metabolism induced by BPH. The study further found that KV, QC and KV+QC administration led to improvements in total bilirubin, total protein, albumin, conjugated bilirubin, and globulin levels, suggesting a potential therapeutic effect on hepatic function associated with BPH induction. Our results are consistent with the findings reported by Farombi et al. (2012).

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) serve as dependable indicators of hepatotoxicity (Edwards, 1997). Elevated AST and ALT levels signify hepatocyte necrosis, whereas ALP levels indicate damage to biliary epithelial cells or the canalicular membrane (Giannini et al., 2005; Padda et al., 2011). This study revealed that BPH induction elevated hepatic levels of AST, ALT and ALP, indicating that BPH pathogenesis adversely affects the integrity of the liver. However, co-administration of

kolaviron and quercetin reversed these increases, reaffirming the report of Kalu *et al.* (2016) as well as Farombi *et al.*, (2012) that both kolaviron and quercetin confer hepatoprotection.

5.0 CONCLUSION

This study showed polyphenols (kolaviron and quercetin) effectively ameliorated hepato-toxicity induced by testosterone propionate overdose by restoring hepatic integrity. Therefore, kolaviron and quercetin may have a similar therapeutic effect against BPH to finasteride, but at a lower cost and with fewer side effects.

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