EFFECT OF POLYPHENOLS (*KOLAVIRON AND QUERCETIN*) ON HEPATIC INDICES OF TESTOSTERONE PROPIONATE INDUCED-BENIGN PROSTATIC HYPERPLASIA IN WISTAR RATS

Ujong, U.P.[1,2], Ibor, M.E.[2] and Peter-Ujong, V[1]

[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Cross River State, Okuku Campus, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Nigeria.

ABSTRACT

This research examined the impact of polyphenols (kolaviron and quercetin) on hepatic indices 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 ALP, AST, ALT, 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: Testosterone propionate, Kolaviron, Quercetin, BPH.

*Corresponding Author
Dr. Ujong Peter Ujong; Department of Medical Biochemistry, University of Cross River State, Nigeria; ujongpeteru@gmail.com, +2348036780699

Citing this article
Ujong, U.P., Ibor, M.E. and Peter-Ujong, V. Effect of Polyphenols (Kolaviron and Quercetin) on Hepatic indices of Testosterone Propionate Induced-Benign prostatic Hyperplasia in Wistar rats. KIU J. Health Sci, 2024: 4(1);

Conflict of Interest: Authors declare that there is no conflict of interest

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) [1]. BPH, a condition associated with aging, entails the non-malignant enlargement and uncontrollable growth of the prostate [2]. 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 [3]. 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 [4].

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 [5]. 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 [6]. Recent studies have shown promising results in hematological and anti-oxidant indices from the use of kolaviron and quercetin in the treatment of BPH [7, 8]. 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.

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 (≥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.

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 [9].

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.

Induction and confirmation of BPH

Thirty-five (35) rats were induced with BPH and seven were not. Induction of BPHT was done using estosterone propionate. 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 [10]. 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.

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.

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.

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 and finally 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.

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.

Statistical analysis

All data were converted to mean ±SEM. Analysis of data was done using Analysis of Variance with GraphPad Prism statistics, version 8.0. Significant differences were proved to exist when the sig. Or p-value was less or equal to 0.05 ($p \leq 0.05$).

RESULTS

The results of organ weight (liver) Fig. 1 show that BHP induction decreases liver tissue weight compared to normal control, while all treated groups (KV, QC, KV+QC) show an increase in liver tissue weight compared to the 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 the BPH group (fig. 2,3,4, and 5).

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.

DISCUSSION

In this study, Testosterone propionate induction reduces the 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 the 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 [11]. Research by Liu et al. (2019) [12] demonstrated that the induction of BPH in Wistar rats resulted in a significant reduction in liver weight in contrast with the 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 [12].

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) [13].

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) serve as dependable indicators of hepatotoxicity [14]. Elevated AST and ALT levels signify hepatocyte necrosis, whereas ALP levels indicate damage to biliary epithelial cells or the canalicular membrane [15, 16]. 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, the co-administration of kolaviron and quercetin reversed these increases, reaffirming the report of Kalu et al., (2016) [17] as well as Farombi et al., (2012) [13] that both kolaviron and quercetin confer hepatoprotection.

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.

REFERENCE

1. Delella F. K & Felisbino S. L. Doxazosin Treatment Alters Stromal Cell Behavior and Increases Elastic System Fibers Deposition in Rat Prostate. *Microsc Res Tech*. 2010; 73:1036–44.
2. Ejike C. E & Ezeanyika L. U. Inhibition of the Experimental Induction of Benign Prostate Hyperplasia: A Possible Role for Fluted Pumpkin (*Telfairia occidentalis* Hook f.) Seeds. *Urol Int.* 2011; 87:218–24.
3. Shin I. S, Lee M. Y, Jung D. Y, Seo C. S, Ha H. K & Shin H. K. Ursolic acid reduces prostate size and dihydrotestosterone level in a rat model of benign prostate hyperplasia. *Food Chem Toxicol.* 2012; 50: 884–8.
4. Sarbishegi M, Khajavi O & Arab M. R. *Withania coagulans* Extract Induces Cell Apoptosis and Inhibits COX-2 Expression in a

Rat Model of Benign Prostatic Hyperplasia. *Nephrourol Mon*. 2016;5: e39284.

5. Adaramoye, O. A., & Lawal, S. O. Kolaviron, a bi-flavonoid fraction from Garcinia kola, protects against isoproterenol-induced injury by mitigating cardiac dysfunction and oxidative stress in rats. *Jour of bas and clini Physiol and pharm*. 2015; *26*(1), 65-72.
6. Gbadamosi, M. R., Abdallah, M. A., & Harrad, S. A critical review of human exposure to organophosphate esters with a focus on dietary intake. *Scie of the Tot Enviro*. 2021; 771, 144752.
7. Ujong, U. P. & Okori, S. O. Polyphenol treatment - a panacea for benign prostatic hyperplasia-triggered immune insults. *Jour of Sci Engin and Techn*. 2023; 10(1): 48-63.
8. Ujong, U. P. (2023). Combined Antioxidant and Hepatoprotective Action of Kolaviron and Quercetin against benign prostrate hyperplasia-triggered oxidative stress and hepatotoxicity in rat models. *Unicr Jour of science and techno*. 2023;123-134.
9. Iwu, M. M. Handbook of African Medicinal Plants. CRC Press: London 1993; 183–184.
10. Gasco, M., Villegas, L., Yucra, S., Rubio, J. & Gonzales, G. F. Dose-response effect of Red Maca (*Lepidium meyenii*) on benign prostatic hyperplasia induced by testosterone enanthate. *Phytomedi*. 2007; 14: 460-464.
11. Fibbi B., Penna, G., Annamaria, M., Adorini, L., & Mario M. "Chronic inflammation in the pathogenesis of benign prostatic hyperplasia." *International Journal of Andrology* 33, no. 3 2010: 475-488.
12. Liu, Y., Wang, Y., Gao, Y., & Zhao, X. Effect of androgen excess induced by testosterone propionate on hepatic lipid metabolism in female Wistar rats. *Fronti in Physiol*, 2019;10, 827.
13. Farombi, E.O., Adedara, I.A., Akinrinde, S.A., Ojo, O.O., & Eboh, A.S. Protective effects of kolaviron and quercetin on cadmium-induced testicular damage and endocrine pathology in rats. *Andro*. 2012 44:273–284.
14. Edwards, G., Marshall, E. J. and Cook, C. C. H. (1997). Drug problems and alcohol problems. In: The treatment of drinking problems- A guide for the helping professions, Third Edition, Cambridge University Press, Cambridge, 110-121.
15. Giannini, E. G., Testa, R. and Savarino, V. (2005). Liver Enzyme alteration: a guide for clinicians. Canadian Medical Association Journal, 172(3): 367-379.
16. Padda, M. S., Sanchez, M., Akhtar, A. J. and Boyer, J. L. (2011). Drug-induced cholestasis. Hepatology, 53(4): 13771387.
17. Kalu, W., Okafor, P., Ijeh, I., & Eleazu, C. Effect of fractions of kolaviron on some indices of benign prostatic hyperplasia in rats: Identification of the constituents of the bioactive fraction using GC-MS. *RSC.* Adv; 6: 2016; 94352–94360.

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 ± 0.606a	33.29 ± 0.606a	155.3 ± 1.848a
BPH + Quercetin	79.57 ± 2.034a	37.00 ± 1.113a	152.1 ± 1.696a
BPH + KV + QC	60.86 ± 0.553a	38.29 ± 1.229	160.6 ± 1.378a
BPH + Finasteride	59.86 ± 0.634a	30.14 ± 0.829a	130.6 ± 1.212a

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 ($p<0.05$).

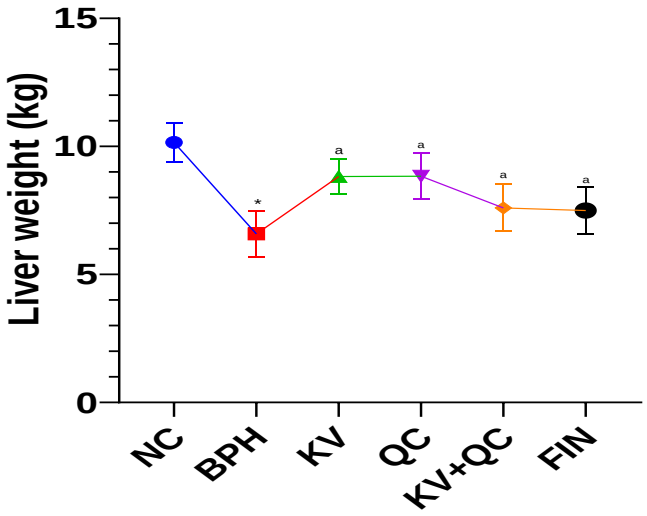

FIG.1: Effect of Kolaviron and quercetin on Liver weight
Values are given as mean ± SEM; n=7.
Significant differences from the control group are denoted by * (p<0.05)
Significant differences from the BPH group are denoted by a (p<0.05)

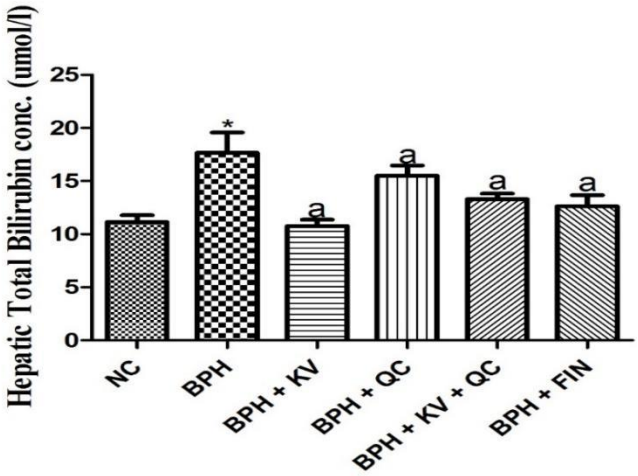

FIG.2: Effect of Kolaviron and Quercetin on Hepatic Total Bilirubin
Values are given as mean ± SEM; n=7.
Significant differences from the control group are denoted by * (p<0.05)
Significant differences from the BPH group are denoted by a (p<0.05)

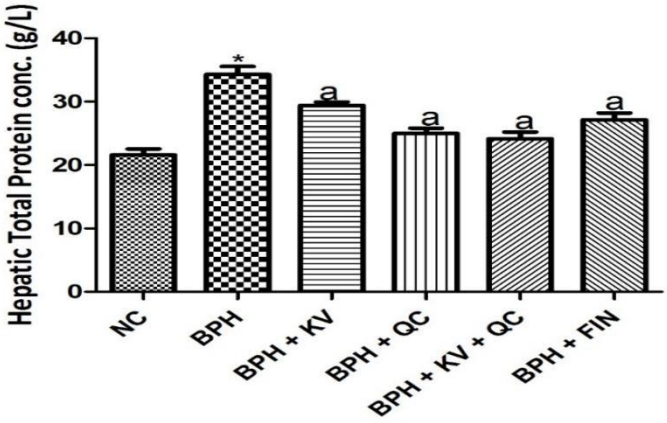

FIG.3: Effect of Kolaviron and quercetin on Hepatic Total Protein
Values are given as mean ± SEM; n=7.
Significant differences from the control group are denoted by * (p<0.05)
Significant differences from the BPH group are denoted by a (p<0.05)

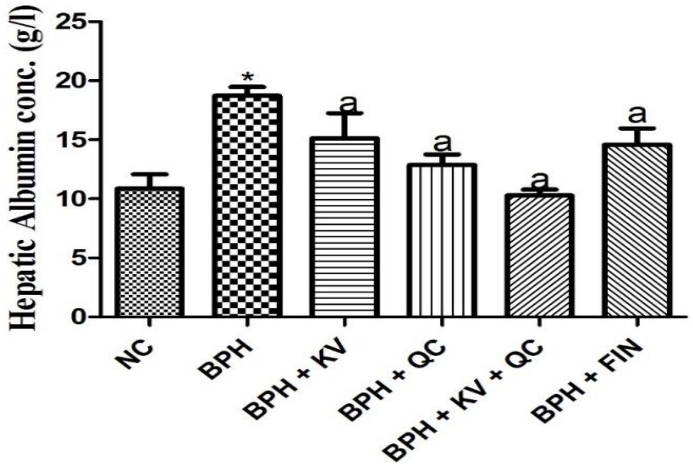

FIG.4: Effect of Kolaviron and Quercetin on Hepatic Albumin
Values are given as mean ± SEM; n=7.
Significant differences from the control group are denoted by * (p<0.05)
Significant differences from the BPH group are denoted by a (p<0.05)

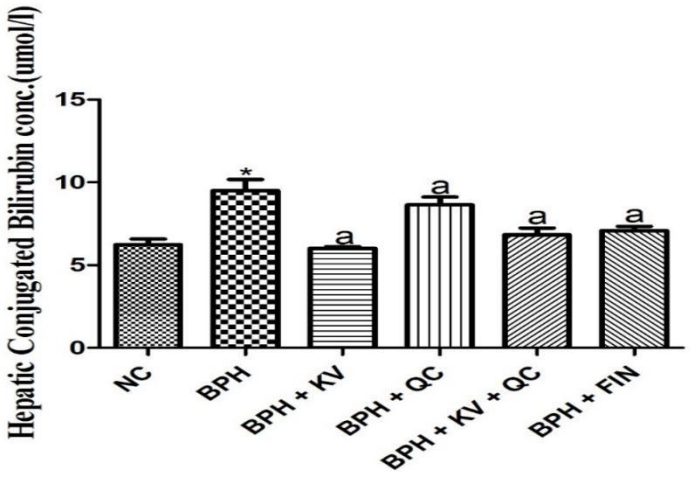

FIG.5: Effect of Kolaviron and Quercetin on Hepatic Conjugated Bilirubin
Values are given as mean ± SEM; n=7.
Significant differences from the control group are denoted by * ($p<0.05$)
Significant differences from the BPH group are denoted by a ($p<0.05$)

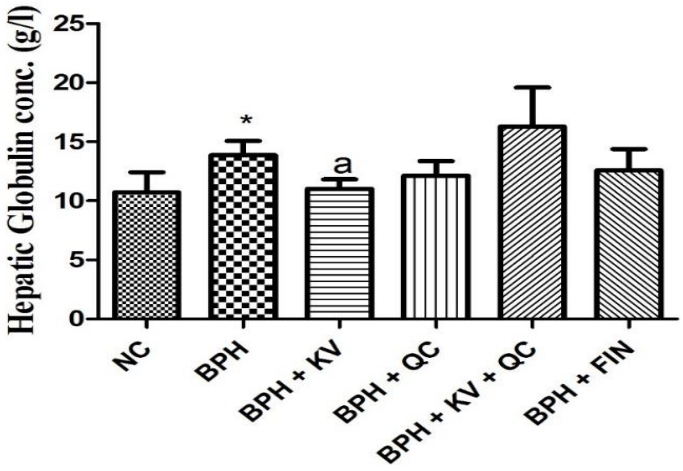

FIG.6: Effect of Kolaviron and quercetin on Hepatic Globulin
Values are given as mean ± SEM; n=7.
Significant differences from the control group are denoted by * ($p<0.05$)
Significant differences from the BPH group are denoted by a ($p<0.05$)