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COMBINED ANTIOXIDANT AND HEPATOPROTECTIVE ACTION OF KOLAVIRON AND QUERCETIN AGAINST BENIGN PROSTATE HYPERPLASIA-TRIGGERED OXIDATIVE STRESS AND HEPATOTOXICITY IN RAT MODELS

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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; usually causing reduction in quality of life. This study investigated the ameliorative effect of polyphenols, Kolaviron and Quercetin on serum liver enzymes and selected antioxidant biomarkers of testosterone-induced benign prostatic hyperplasia (BPH) 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 centrifuged, the serum gotten was used for biochemical assays. Testosterone induction increased the levels of ALP, AST and ALT significantly compared with the normal control group. Treatments with polyphenols, kolaviron and quercetin reversed the increases observed when compared with normal control group. Furthermore, testosterone induction caused significant decrease in levels of antioxidant enzymes SOD, CAT, GSH, GST and TAC compared with normal control, while causing significant increase in GPx levels. Treatments with polyphenols, kolaviron and quercetin reversed the observed biochemical insults by restoring antioxidant statuses, when compared with normal control group. Conclusively, the results suggest that co-administration of polyphenols like kolaviron and quercetin could be a promising treatment in ameliorating BPH-induced oxidative stress and hepatocellular toxicity.

1.0 Introduction

Nature has endowed mankind with manya variety of edible plants; although most of such plants indigenous to developing nations like Nigeria, are grossly neglected. Medicinal plants have been used for various traditional purposes varying from one community to another. Majority of people especially from the developing countries, rely on traditional plant usage for their day-to-day health care needs,[5, 3] mostly due to the high cost of conventional health care or side effects of drugs used.

Polyphenols, such as kolaviron and quercetin, have been proposed as an alternative to convensional orthodox treatment for BPH[15, 20]. Kolaviron is a biflavonoid complex from Garcinia kola, while quercetin is a flavonoid found in many fruits and vegetables. Both compounds have been reported to have anti-inflammatory and anti-proliferative effects on prostate cells, making them potential candidates for the treatment of BPH[8]. Recent studies have shown promising results in haematological indices from the use of kolaviron and quercetin in the treatment of BPH[21]. 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[10,2].

The prostate is a gland of the male reproductive system. In adults, it is about the size of a walnut [22] and has an average weight of about 11 grams, usually ranging between 7 and 16 grams[11].The prostate is located in the pelvis. It sits below the urinary bladder and surrounds the urethra. The part of the urethra passing through it is called the prostatic urethra, which joins with the two ejaculatory ducts[22]. The changes in the shape of the prostate facilitate the mechanical switch between urination and ejaculation, which are mainly driven by the two longitudinal muscle systems running along the prostatic urethra. These are the urethral dilator (*Musculus dilator urethrae*) on the urethra's front side, which contracts during urination and thereby shortens and tilts the prostate in its vertical dimension thus widening the prostatic section of the urethral tube[4] and the muscle switching the urethra into the ejaculatory state (*Musculus ejaculatorius*) on its backside. In case of surgery because of benign prostatic hyperplasia (BPH), damaging or sparing of these two muscle systems varies considerably depending on the choice of surgery type and details of the procedure of the chosen technique. The effects on post operational urination and ejaculation vary correspondingly[12].

2.0 Materials and methods

Testosterone propionate manufactured by Greenfield Pharma, Jiangsu, China, wasbought 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 was purchased from Fidelity Medical Store, Igoli-Ogoja, Cross River State, Nigeria.

2.1 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 40–60oC) 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), 13C NMR, and negatron ionization (EI)-mass spectral results with antecedently revealed knowledge[9].

2.2 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^{o}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.

2.3 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 [7] 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.

2.4 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.

2.5 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. After a twelve hour fast, all animals were sacrificed under chloroform anaesthesia, and blood samples were collected for biochemical analysis.

2.6 Biochemical analysis

Total antioxidant capacity, glutathione-s-transferase, calatase, superoxide dismutase, glutathione, glutathione peroxidase, alkaline phodphatase, alanine amino transferase, and alkaline transaminasewere measured in serum using kits purchased from Randox Laboratories Limited, UnitedKingdom (UK) following the manufacturer's protocols.

2.7 Statistical analysis

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

3.0 Results

Table 2: Effect of Kolaviron and Quercetin treatment on liver function enzymes in serum.

Group	Serum AST conc. (IU/L)	Serum ALT conc. (IU/L)	Serum ALP conc. (IU/L)
Normal control	65.29 ± 0.865	53.00 ± 1.069	134.1 ± 1.639
BPH control	121.6 ± 1.429*	95.71 ± 0.565*	211.7 ± 3.183*
BPH + Kolaviron	87.14 ± 1.033a	77.00 ± 0.926a	173.1 ± 1.550a
BPH + Quercetin	98.86 ± 1.805a	87.43 ± 2.170a	188.6 ± 1.043a
BPH + KV + QC	86.86 ± 0.911a	71.29 ± 1.286a	153.9 ± 2.586a
BPH + Finasteride	75.71 ± 0.837a	65.14 ± 0.340a	140.0 ± 1.069a

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 ($p<0.05$)

3.1 Effect of kolaviron and quercetin treatment on liver function enzymes in serum

The results for liver function enzymes, AST, ALT and ALP in serum (Table 2) showed that there were significant ($p<0.05$) increases in the levels of these enzymes in sera of the BPH group when compared with the normal control group. All the treatment groups (KV only, QC only, and co-treatment with KV and QC) recorded significant ($p<0.05$) decreases in their concentrations of AST, ALT and ALP, when compared with the BPH group.

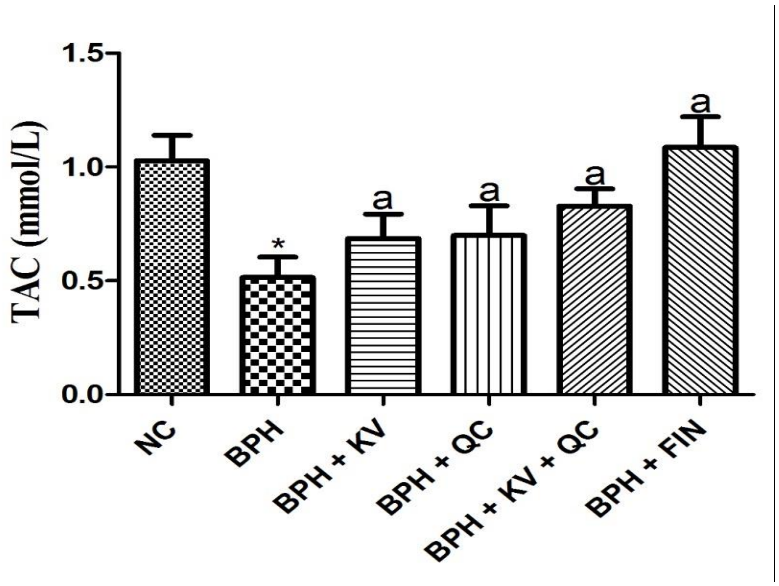

FIG. 1: Effect of Kolaviron and quercetin on Serum TAC concentration
Values are given as mean ± SEM; n=7.
Significant differences from the control group are denoted by * ($p<0.05$)
Significant differences from the BPH group are denoted by a ($p<0.05$)

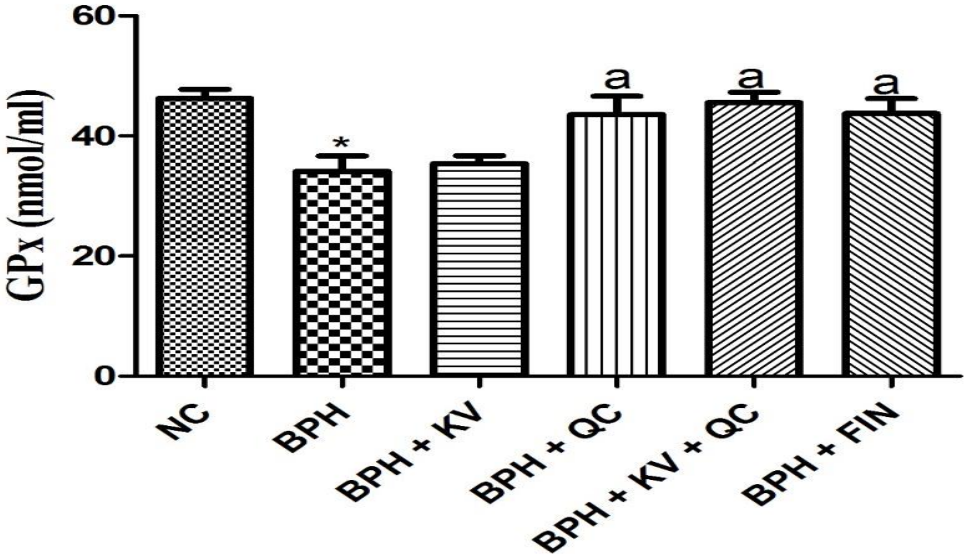

FIG.2: Effect of Kolaviron and quercetin on Serum GPx concentration
Values are given as mean ± SEM; n=7.
Significant differences from the control group are denoted by * ($p<0.05$)
Significant differences from the BPH group are denoted by a ($p<0.05$)

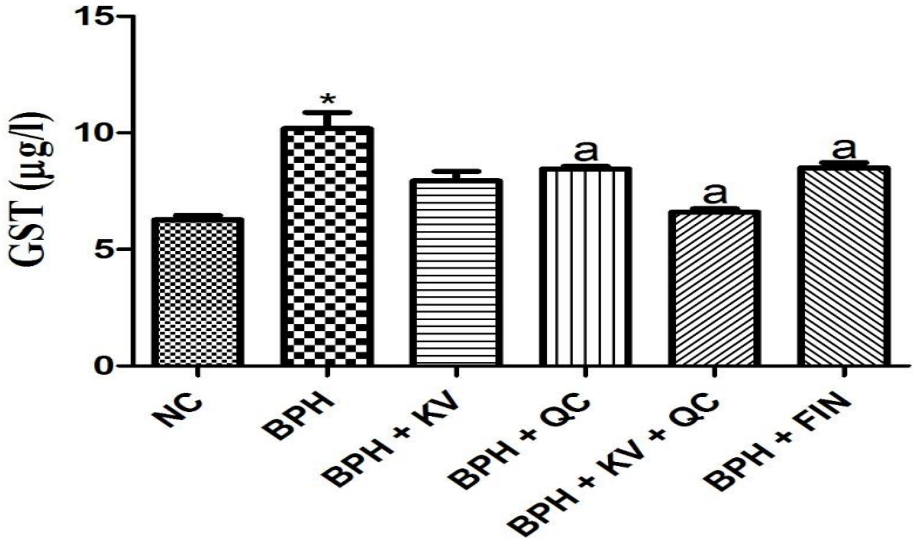

FIG.3: Effect of Kolaviron and quercetin on serum GST concentration
Values are given as mean ± SEM; n=7.
Significant differences from the control group are denoted by * ($p<0.05$)
Significant differences from the BPH group are denoted by a ($p<0.05$)

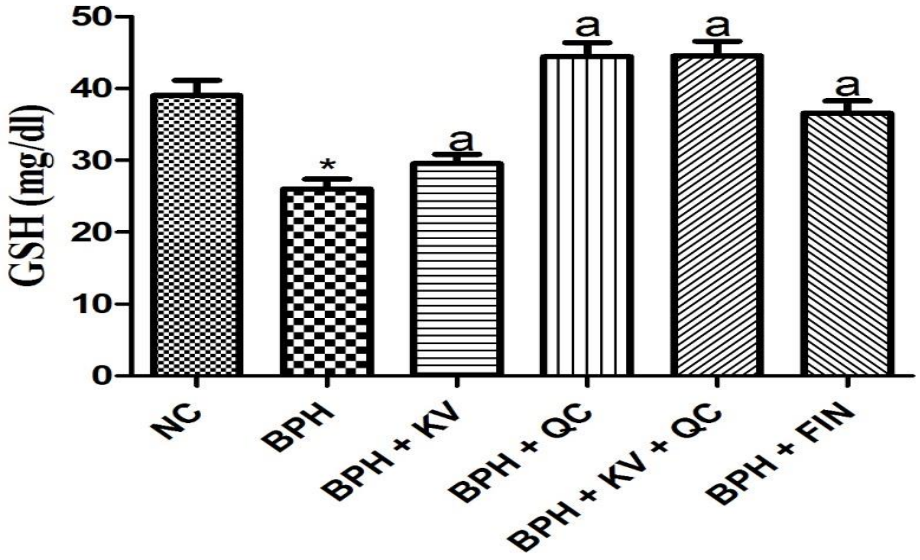

FIG.4: Effect of Kolaviron and quercetin on serum GSH concentration
Values are given as mean ± SEM; n=7.
Significant differences from the control group are denoted by * ($p<0.05$)
Significant differences from the BPH group are denoted by [a] ($p<0.05$)

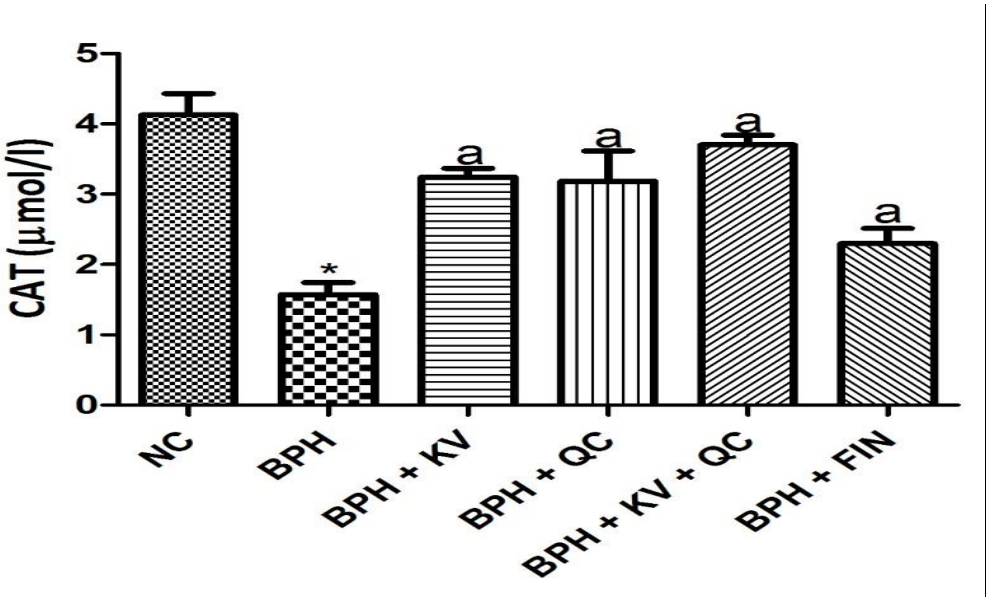

FIG.5: Effect of Kolaviron and quercetin on serum CAT concentration
Values are given as mean ± SEM; n=7.
Significant differences from the control group are denoted by * ($p<0.05$)
Significant differences from the BPH group are denoted by [a] ($p<0.05$)

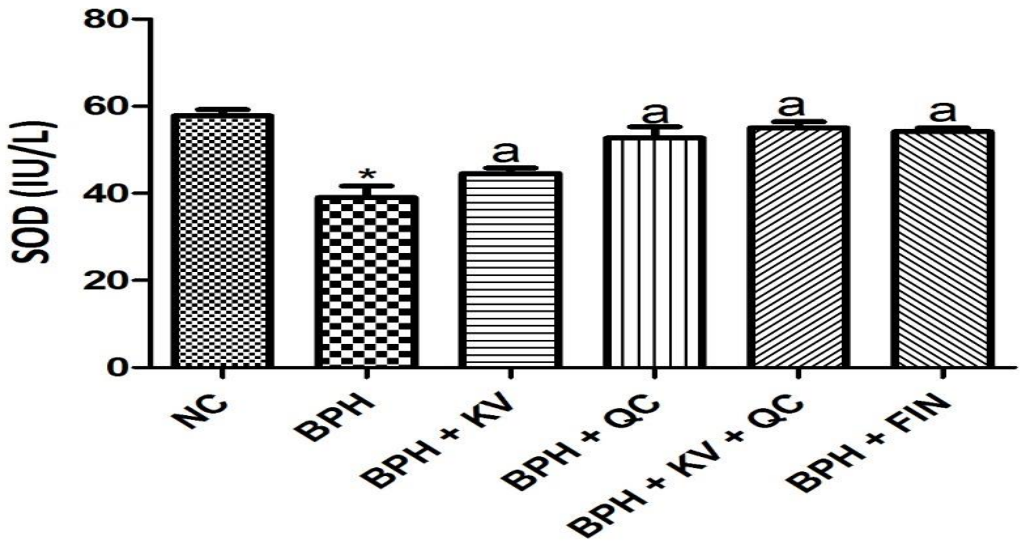

FIG.6: Effect of Kolaviron and quercetin on serum SOD concentration
Values are given as mean ± SEM; n=7.
Significant differences from the control group are denoted by * ($p<0.05$)
Significant differences from the BPH group are denoted by a ($p<0.05$)

3.2 Effect of kolaviron and quercetin on antioxidant enzymes

The results for SOD, CAT and GSH, all show that there were significant ($P<0.05$) decreases in the levels of these respective enzymes in the sera of group II (BPH) rats when compared with the normal control, while the groups respectively treated with KV, QC, and the group co-treated with KV and QC, all showed significant ($P<0.05$) increases in levels of SOD, CAT and GSH in serum samples, when compared with the BPH group (Figures 4,5,6).

3.3 Effect of kolaviron and quercetin on serum GST concentration

The results for GST in serum samples (Fig. 3) show there was a significant ($P<0.05$) increase in GST levels of the BPH group, when compared with the normal control, while the groups treated with KV, QC and a combination KV and QC all recorded significantly ($P<0.05$) decreased GST levels, when compared with the BPH group.

3.4 Effect of kolaviron and quercetin on antioxidant statues

The results for GPx and TAC in serum (Figures 1, 2) show that there were significant ($P<0.05$) decreases in GPx and TAC levels of the BPH group when compared with normal control, while the groups treated with KV, QC, as well as the group cotreated with KV and QC all recorded significant ($P<0.05$) increases in GPx and TAC.

4.0 Discussion

Benign prostatic hyperplasia (BPH) is a pathologic condition that is common among men over forty years of age. The incidence

and progression of BPH increases according to the age of men. It represents a common health problem, resulting in significant morbidity and associated patient care costs. [19,18]

BPH is considered to be a product of androgen action upon an aging prostate; however, longitudinal epidemiological studies have shown that androgen levels are unlikely to be solely responsible. The exact pathogenesis of BPH is still unclear;[17] several clinical and experimental observations indicate also that oxidative stress and inflammation are closely associated with the progression of BPH.

Acute and chronic inflammation contribute to the development of BPH by stimulating cellular growth through various pathways particularly oxidative stress[16]. Although controlled production of reactive oxygen species (ROS) is necessary for cellular signaling, proliferation, apoptosis, and protection against microorganisms, higher concentrations are associated with a variety of diseases including BPH.[20] Prostate tissue is normally protected from oxidative damage by free radical scavengers and enzymatic antioxidants such as glutathione, catalase, and superoxide dismutase[13].

In this study, BPH induction elevated serum levels of AST, ALT and ALP, indicating that BPH pathogenesis adversely affects the intergrity 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 hepotoprotection.

This study further assessed free radical scavenging enzymes in serum of testosterone-induced BPH rats. BPH induction resulted to decreases in serum levels of CAT, SOD, as well as GSH, this depletion was linked to BPH and its accompanying generation of reactive oxygen species which led to the reduction in antioxidant capacity of the prostatic cells.
Treatment with the polyphenols kolaviron and quercetin, both singly and in combination reversed these decreases. This might have been due to the free radical scavenging activities of kolaviron and quercetin which is an indication of the antioxidant activities of kolaviron and quercetin against BPH-induced oxidative stress. The findings are in synchrony with earlier reports by Adedara and Farombi (2012) who reported the existence of a mutually supportive relationship between SOD and CAT against the accumulation of reactive oxygen species (ROS) which inactivate the superoxide anion and peroxide radicals by converting them into water and oxygen. The findings also suggested an inhibition of enzymes involved in antioxidant defence mechanism against free radicals generated following BPH induction in rats. The results corroborated with previous observation on the effect of BPH induction on antioxidant enzymes.[14] However, administration of kolaviron and quercetin reversed the decrease in the activities of these antioxidant enzymes, thus suggesting kolaviron and quercetin exhibited their ameliorative effect against BPH by enhancing the antioxidant enzymes probably through their free radical scavenging

activities and having potential of protecting endogenous antioxidant enzymes.

Glutathione S-transferase (GST) is directly responsible for the elimination of electrophilc oxidants at the expense of GSH. This study demonstrated that BPH induction increased GST in both serum and liver. This observation possibly suggested that the detoxification process mediated by this enzyme was induced along with a corresponding utilization of GSH, hence the depletion in the levels of GSH recorded. These results revealed that kolaviron and quercetin are capable of breaking lipid peroxidation chains and shielding the membrane from free radical-mediated injuries in the prostate. Antiperoxidative effect of kolaviron may be linked to the presence of a C-4 carbonyl and C-5 and C-7 hydroxyl groups of the A-ring,[6] whereas the hydroxyl groups at 3 and 4-positions in the B-ring and 3-position in the C-ring are responsible for the high reactivity of quercetin with free radicals.[14]

Conclusion

The results of this study suggest that co-administration of kolaviron and quercetin could be a promising treatment in ameliorating BPH-induced oxidative stress and hepatocellular toxicity.

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