OXIDATIVE STRESS MARKERS IN GONADAL TISSUES OF RABBITS SUPPLEMENTED WITH CRUDE EXTRACT OF BITTER KOLA (*Garcinia kola*) SEED IN WATER

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ABSTRACT
During a 32–week study, the oxidative stress markers in the gonadal tissues of rabbits supplemented with bitter kola seed crude extracts (BKSCE) in drinking water was investigated at the Rabbitry unit, Livestock Teaching and Research Farm, Joseph Sarwuan Tarka University, Makurdi. Fifty-four (54) nondescript rabbits (both sexes), aged 9–10 weeks were assigned randomly to 3 groups comprising 18 rabbits each that was replicated 6 times with 3 rabbits per replicate in a 2x3 factorial of a Completely Randomized Design. Fresh bitter kola seeds were processed and extracted by macerating 1500 g powder per 10 L water for 24 hours followed by sieving and cold storage until used. The stored extract was reconstituted at 0, 25 and 50% levels respectively and offered daily at a single dose (100 ml) in drinking water. Rabbits received accordingly the basal diet and 3 levels of the reconstituted extract. The oxidative stress markers (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GTP_x; and malondialdehyde, MDA) in the gonads of 36 sacrificed rabbits (18 per sex) were determined accordingly using tissue assays. Data were subjected to General Linear Model using IBM SPSS version 21 with significance denoted at probability of $P<0.05$. BKSCE significantly ($P<0.05$) increased the testicular concentration of MDA thereby favouring lipid peroxidation while SOD, CAT and GTPx were not significant ($P>0.05$). Also, all the oxidative stress markers in the ovarian tissue were not influenced ($P>0.05$) by BKSCE. In conclusion, BKSCE most likely induced testicular lipid peroxidation of rabbits.
Keywords: Antioxidants, Crude extracts, *Garcinia kola*, Gonads, Micro livestock

INTRODUCTION

Oxidative stress results from imbalances between free radicals and antioxidants within the body with the former causing damages to cell structures and tissues when in excess while the latter inhibits the former's damage–causing ability. Oxidative stress can affect body tissues and organs such as the gonads. The male reproductive system has limited efficient antioxidant capacity and is thus highly susceptible to lipid peroxidation (Halliwell and Gutteridge, 2015). Oxidative stress is mainly defended against by endogenous antioxidants (Abolaji *et al*., 2015) and repair enzymes (Oboh *et al*., 2018). Antioxidants are important in destroying free radicals generated due to several conditions, particularly high ambient temperature associated with tropically–raised animals (Jimoh *et al*., 2017). However, endogenous enzymatic antioxidants such as superoxide dismutase, catalase and glutathione peroxidase usually occur in small amounts in relation to rapidly increasing free radicals, thus, the need to augment with exogenous sources from antioxidant–rich plants such as bitter kola (*Garcinia kola*). *G. kola* tree has varying medicinal uses of all of its parts (Onasanwo and Rotu, 2016) and the seed is the most valued (Manourova *et al*., 2019) with antioxidant properties and low anti-nutritive levels (Onyekwelu *et al*., 2015). Literature is very limited on oxidative stress markers in gonadal tissues of rabbits, particularly of those exposed to phytonutrients of bitter kola seed (BKS) thus prompting this research interest. Therefore, the oxidative stress markers in the gonadal tissues of rabbits supplemented with bitter kola seed crude extracts (BKSCE) in drinking water was investigated in this study.

MATERIALS AND METHODS

Experimental site

The study was conducted (within January–June, 2024) at the rabbitry unit of the Livestock Teaching and Research Farm, Joseph Sarwuan Tarka University, Makurdi, Benue state, Nigeria. Makurdi falls within latitude 7˚33' 00"N–7˚47' 00"N and longitude 8˚27'00" E–8˚4'00"E.

Sourcing, processing and preparation of bitter kola seed crude extract (BKSCE)

Bitter kola seeds (BKS) were sourced from a local market in Enugu state then processed (sorted, sliced, crisp–dried, dehulled and milled) and the powder stored in an air tight container. Extraction was done by macerating 1500g milled BKS per 10 L mildly hot water (50–55°C) for 24 hours followed by sieving and refrigeration (4–5°C) until used.

Experimental animals, design and management

A total of 54 weaned nondescript rabbits (27 males and 27 females), aged 9–10 weeks were randomly assigned after equalizing for weight to 3 groups comprising 18 rabbits each i.e. 9 rabbits per sex. Each group was replicated

6 times with 3 rabbits per replicate in a 2x3 factorial of a Completely Randomized Design. The rabbits were housed singly in galvanized wire and mesh cages under strict management practices and acclimated for 2 weeks prior to start of trial. The stored BKSCE was reconstituted at 0, 25 and 50 % levels respectively for inclusion in the drinking water of rabbits. The rabbits irrespective of their grouping received a formulated basal diet (moisture 5.34%, crude protein 17.06%, crude fibre 10.23%, crude fat 8.52%, ash 19.98%, energy 3530 kcal, lysine 0.94%, methionine 0.60%, calcium 0.52% and phosphorus 0.33%). The basal diet contained maize (25.51%), full fat soya bean (20.00%), soya bean cake (7.94%), brewers dried grain (20.00%), rice offal (22.00%), unrefined soya oil (2.70%), bone ash (0.55%), limestone (0.50%), methionine (0.30%), salt (0.25%) and vitamin premix (0.25%). The reconstituted levels of BKSCE were offered daily as a single dose (100 ml) in drinking water based on estimated drinking rate at 10 % body weight (Brewer and Cruise, 1994; Harcourt–Brown, 2002). The study lasted for 32 weeks.

Parameters and data analysis

At week 32 (aged 33–34 weeks), a total of 36 rabbits (2 per replicate and 18 per sex) were sacrificed by neck slaughter and the testes and ovaries excised while tissue assays were used to determine the oxidative stress markers (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GTPx; and malondialdehyde, MDA) according to standard procedures. Data were subjected to General Linear Model at 5% significance ($P<0.05$) using IBM SPSS version 21 while significance was detected at probability of $P<0.05$. Significant means were separated where applicable using Fisher's Least Significant Difference (LSD).

RESULTS AND DISCUSSION

The oxidative stress markers in the testicular tissues of rabbits supplemented with BKSCE in water is presented in table 1. The results showed that SOD, CAT and GTPx were not affected ($P>0.05$) by the levels of BKSCE even though numerically higher values were obtained by 25% BKSCE. However, MDA showed a significant ($P<0.05$) increase in accordance with the BKSCE levels. The numerically higher SOD, CAT and GTPx values recorded by 25% BKSCE could be an indication of increased antioxidant status. Jimoh (2019) opined that the proliferation of free radicals intensified tissue synthesis of antioxidant enzymes hence their elevated activity a likely manifestation of adaptative mechanisms in response to oxidative stress. In addition, Seven *et al*. (2001) reported that reductions in the activity of antioxidant enzymes or concentration of non-enzymatic antioxidants may be caused by their intensified utilization in protection against oxidative tissue damage. The results of SOD, CAT and GTPx in this study were at variance with the significant differences observed by Jimoh (2024) for testicular oxidative stress markers of rabbits fed herbal supplements of *Moringa oleifera* (drumstick), *Viscum album* (mistletoe) and *Phyllanthus amarus* (carry me seed) leaf meals. However, this author observed significant elevations in testicular MDA which also concurred with the findings of this study. This infers that BKSCE in combination with other influences may have caused reductions in the antioxidant status thereby inducing testicular lipid peroxidation. Literature on the gonadal markers of rabbits fed phytonutrient herbs is generally very scarce thereby limiting adequate comparisons that could have been made.

Table 1: Oxidative Stress Markers in Testicular Tissues of Rabbits Given Bitter Kola Seed Crude Extract in Water

	BKSCE Levels				
Parameters	**0 % (n=6)**	**25 % (n=6)**	**50 % (n=6)**	**± SE**	**P-value^{LOS}**
SOD (U/L)	15.56	17.77	15.89	5.21	0.949^{ns}
CAT (U/L)	272.26	454.09	426.46	66.27	0.147^{ns}
GTPx (U/L)	0.61	1.02	0.96	0.15	0.150^{ns}
MDA (mmol/L)	14.78^{bc}	22.02^{a}	25.68^{a}	2.06	0.006*

^{a,b,c} Means in the same row with different superscripts differ significantly; SE = standard error; BKSCE = bitter kola seed crude extract; n = sample size; LOS = level of significance; ns = not significant ($P>0.05$);
* = significant ($P<0.05$); SOD = superoxide dismutase; CAT = catalase; GTPx = glutathione peroxidase; MDA = malondialdehyde

The SOD, CAT and MDA values in this study were higher than those reported by Jimoh (2024) for testicular oxidative stress markers of rabbits while GTPx values in this study were lower than that obtained by the same author. The observed differences between values of these oxidative stress markers studied and those reported by the previous author could be attributed to variations in the quantification methods, nutrition, management conditions, ambient temperature, physiological age etc. Table 2 presents the oxidative stress markers in the ovarian tissues of rabbits supplemented with BKSCE in water. BKSCE did not significantly ($P>0.05$) influence any of the oxidative stress markers, even though 0% BKSCE recorded numerically higher values for SOD, CAT and GTPx. Jimoh (2019) opined that low SOD anion status resulted in its reduced ability to scavenge the free radical superoxide anions which accumulate thereby accounting for high lipid peroxidation and subsequent oxidative

stress in animals. The statistical similarity and concomitant higher values of SOD, CAT and GTPx in ovarian tissues recorded by 0% BKSCE, suggest the minimal exposure of the control group to oxidative stress with regards to the extract as opposed to the other groups which experienced the apparent decline in antioxidant status due to intensified antioxidant utilization against oxidative damage.

Table 2: Oxidative Stress Markers in Ovarian Tissues of Rabbits Given Bitter Kola Seed Crude Extract in Water

	BKSCE Levels				
Parameters	**0 % (n=6)**	**25 % (n=6)**	**50 % (n=6)**	**± SE**	**P-value^{LOS}**
SOD (U/L)	22.55	9.87	16.81	4.68	0.193^{ns}
CAT (U/L)	607.38	359.61	495.87	95.15	0.216^{ns}
GTPx (U/L)	1.37	0.81	1.12	0.22	0.215^{ns}
MDA (mmol/L)	23.31	31.73	24.38	3.93	0.287^{ns}

Means in the same row without superscripts do not differ significantly; SE = standard error;
BKSCE = bitter kola seed crude extract; n = sample size; ^{LOS} = level of significance; ns = not significant (P>0.05);
SOD = superoxide dismutase; CAT = catalase; GTPx = glutathione peroxidase; MDA = malondialdehyde

CONCLUSION

In conclusion, BKSCE increased testicular MDA concentrations which possibly induced lipid peroxidation due to oxidative stress while the oxidative stress markers in ovarian tissues were apparently not influenced by BKSCE. Further studies are recommended to validate the results of this study.

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