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Oxidative stress markers in brain and gonads of rabbit bucks fed herbal supplements

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Abstract

Background Currently, there is an increase in the usage of phytogenic feed additives to help improve animal welfare and productivity, while less emphasis is now placed on metabolic and oxidative stability of neuronal and testicular tissues. This study aims at investigating the effects of *Moringa oleifera*, *Phyllanthus amarus* and *Viscum album* as feed additives on some metabolic indicators and oxidative status of rabbit testis and brain. Isonitrogenous and isocaloric diets were formulated with 5% supplementation of each of the phytogenic additive to 3 treatment groups and basal diet group.

Results It was revealed that moringa, mistletoe, and phyllanthus can modulate oxidative status in both the brain and gonads of rabbit bucks through their unique phytochemical compositions, thereby affecting reproductive and cognitive functions. Moringa, rich in crude protein, saponins, glycosides, and steroids, enhances protein and lactate dehydrogenase levels but increases lipid peroxidation in the testis. Mistletoe, with high crude fiber, ash, and antioxidants like flavonoids and tannins, boosts total antioxidant activity in several brain regions and reduces lipid peroxidation, indicating its potential for reducing oxidative stress. Phyllanthus, having the least fiber and ash but effective antioxidant properties, notably affects the oxidative balance in both the testis and brain, with varied impacts on different tissues. The result obtained showed that total antioxidant activity of the left testis was enhanced ($p < 0.05$) by inclusion of the phytogenic additives, while total antioxidant activity of the right testis in bucks fed on phytogenic additives were similar ($p > 0.05$) to bucks on basal diet. Bucks fed on moringa and phyllanthus additives had higher ($p < 0.05$) testicular lipid peroxidation, lowered testicular protein and/or lactate dehydrogenase. Result also shows that lipid peroxidation of hypothalamus, cerebrum, olfactory lobe and cerebellum were lowest in bucks fed on mistletoe, phyllanthus, phyllanthus and phyllanthus, respectively. High catalase activity of optic lobe, olfactory lobe and cerebellum were observed in bucks fed on mistletoe, moringa and mistletoe, respectively, while glutathione peroxidase activity in hypothalamus, cerebrum, olfactory lobe and pineal was enhanced ($p < 0.05$) in bucks fed on moringa and mistletoe compared to bucks on other treatment.

Conclusion *M. oleifera*, *P. amarus* and *V. album* leaves as phytogenic feed additives in rabbit diets have negative effect on the metabolic activity of the testis, enhancing antioxidant activity in the brain.

Keywords Antioxidants, Brain, Lactate, Lipid peroxides, Rabbit bucks, Testis

Background

Oxidation is essential in both gametic and somatic cells for the provision of energy for vital functions, 98% oxygen consumed during aerobic metabolism is reduced to water, while the rest is converted to reactive oxygen species (Jimoh and Ewuola, 2019). A decrease in antioxidant activity (enzymatic or non-enzymatic antioxidant) may arise from intensified utilization in guarding against

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oxidative tissue damage (Jimoh, 2019). The importance of antioxidant system in destroying oxygen free radicals generated due to several conditions, particularly in animals being raised under high ambient temperature of the tropics (Jimoh et al., 2017) is a budding area of research. Male reproductive system and central nervous system are more vulnerable to oxidative damage due to twin issues of low polyunsaturated fatty acid content, which is highly susceptible to lipid peroxidation and limited efficiency of their antioxidant system (Halliwell and Gutteridge, 2015). The biochemical and physiological activities of the brain, coupled with its metabolic requirement (high lipid content and energy requirements), render it susceptible to free radicals induced assault, due to continual generation of reactive oxygen species (Bellés et al., 2010). Testes have lower blood flow and the partial pressure of oxygen, due to its poor vascularization, coupled with spermatogenesis and Leydig cell steroidogenesis processes it runs, which have high rates of mitochondrial oxygen consumption by the germinal epithelium (Tothova et al., 2013) and are more susceptible to oxidative stress than other tissues.

The use of medicinal plants is becoming an object of increasing interest due to its food safety, as the use of antibiotics is banned due to residues in animal products and development of antibiotic resistance (Daramola et al., 2020a). Fortification of diets with herbs rich in natural antioxidants to help mitigate the accumulation of free radicals in the animal's body, which have negative effects on animal production is becoming a popular practice (Oloruntola et al., 2020). There has been heightened search for safe and effective natural antioxidants from herbs and functional foods because they present negligible or no side effects (Daramola et al., 2020b). Utilization of leaf meal as phytogenic growth promoter in animal production is on the increase due to their antioxidant and anti-inflammatory ability to suppress the metabolism of inflammatory prostaglandins (Jimoh et al., 2018c). Some alternatives to synthetic growth promoters and antioxidants (*moringa leaf meal, mistletoe, pawpaw, mucuna, Phyllanthus amarus*) have been evaluated as phytochemical feed additives, phytogenic feed supplements and phytobiotic (Ewuola et al., 2012a, 2012b; Jimoh et al., 2018b; Oloruntola et al., 2020, Jimoh et al., 2020).

Increasing antioxidant defence in different tissues via exogenous antioxidant substrate and/or its precursors requires investigation (Jimoh et al., 2018a). Studies by Pasko et al. (2011) suggest that dietary factors play a vital role in the protection of organs against oxidative stress; however, further research is needed to verify this hypothesis. Thus, it is important to evaluate the oxidative stability in vital organs (different regions of the brain and testis) because any overdosed or incorrectly used therapeutic agent have the potential to induce adverse effects.

Hence, there is a need for comparative assessment of the herbs (*Moringa oleifera, Phyllanthus amarus* and *Viscum album*) as feed additives on oxidative stability of the testis and different regions of rabbit brain.

Methods

Experimental animals and management

This study was undertaken with the approval of the institutional ethics committee for care and use of animals for research and in line with NIH guideline. *Moringa oleifera, Phyllanthus amarus* and *Viscum album* leaves used for this experiment were harvested within the teaching and research farm of the Federal Polytechnic Ado-Ekiti, Ekiti State, Nigeria. Fresh leaves were harvested and shade-dried until crispy to touch and grinded; thereafter, they were referred to as moringa, phyllanthus and mistletoe, respectively, and were analyzed for proximate analysis and phytochemical screening according to AOAC (2010).

Forty-eight (48) mixed breed (Chinchilla X CaliforniaX New Zealand white) rabbit at 6–8 weeks weighing 775 ± 54.23 g were used for the experiment. The bucks were randomly assigned to four (4) dietary treatment groups consisting of twelve animals each, in a completely randomized design. Experimental diets were formulated to meet the nutrient requirement of growing rabbits and were fed to bucks at 4% body weight. The experimental treatment diets are shown in Table 1: T1 basal diet without leaf meal, T2 basal diet with 5% Moringa, T3 basal diet with 5% Mistletoe and T4 basal diet with 5% Phyllanthus.

Testicular tissue preparation and oxidative stress assay

At the end of the 12 weeks of feed trial, all bucks were killed, all bucks were euthanized by cervical dislocation resulting in immediate unconsciousness and death was confirmed by cessation of circulation, after which the pairs of testes were carefully separated and freed of all connective tissues. They were separated into right and left testes and were homogenized in phosphate buffer for biochemical assay. Testicular homogenates were centrifuged at 20,000RPM for 30 min for the assay of antioxidant enzymes, lactate dehydrogenase, total protein, total antioxidant activity and lipid peroxidation.

Brain tissues preparation and oxidative stress assay

The skull of each buck was carefully opened and their brains excised. The whole brain was removed within one minute and dissected into optic lobe, pineal, hypothalamus, cerebrum, olfactory lobe, and cerebellum and immediately stored at − 80 °C. The brain regions were homogenized in 10% w/v cold potassium phosphate buffer (50 mM: pH 7.4). The homogenate was centrifuged at 20,000 RPM for 30 min (Ewuola and Bolarinwa 2017)

Table 1 Gross composition of experimental diets (g/100 g)

Ingredient (%)	Basal diet	Moringa-inclusive diet	Mistletoe-inclusive diet	Phyllanthus-inclusive diet
Rice bran	6	1	1	1
Salt	0.25	0.25	0.25	0.25
Wheat offal	5	5	5	5
Brewers dry grain	5.0	0	0	0
Grower premix	0.25	0.25	0.25	0.25
Maize	25	25	25	25
Methionine	0.40	0.40	0.40	0.40
Soybean meal (44%)	17.0	17.0	17.0	17.0
Lysine	0.10	0.10	0.10	0.10
Bone meal	1.0	1.0	1.0	1.0
Groundnut Hauluns	40.0	40.0	40.0	40.0
MoLM	0	10	0	0
MiLM	0	0	10	0
PLM	0	0	0	10
Vegetable oil	0.20	0.20	0.20	0.20
Total	100	100	100	100
Calculated nutrient composition				
Dry matter %	88.05	85.41	81.2	87.45
Crude protein %	16.47	15.58	17.94	17.667
Digestible energy kcal/kg	2721.6	2515.35	2479.6	2509.4
Ether extract %	3.344	3.022	3.754	2.419
Crude fiber %	16.5	17.325	15.378	16.32
Lysine %	1.0085	0.9385	0.9385	0.9385
Methionine %	0.7482	0.7162	0.7162	0.7162
Calcium %	1.5999	1.7479	1.7159	1.5979
Phosphorus %	0.4491	0.5181	0.5211	0.4211
Proximate composition of experimental diets				
Dry matter %	92.28	92.68	92.55	92.17
Crude protein%	17.50	20.56	19.25	17.19
Ether extract %	5.10	5.50	5.20	5.20
Crude fiber %	13.90	12.10	12.50	14.20
Ash	5.50	5.20	5.40	5.10
Nitrogen free extract	58.00	56.64	57.65	59.31

for the assay of antioxidant enzymes, total protein, total antioxidant activity and lipid peroxidation.

The protein and lactate dehydrogenase content of the samples was determined using fortress diagnostics limited UK commercial assay kits with product codes: BXC0173 and BXC0242, respectively. The activities of antioxidant enzymes, catalase, superoxide dismutase and glutathione peroxidase, total protein, total antioxidant activity and lipid peroxidation were carried out as outlined in Jimoh (2019).

Statistical analysis

The statistical model applied is as follows:

$$Y_{ijl} = \mu + B_i + e_{ijl}$$

where Y_{ijl} represents the value of testicular and brain oxidative stress markers, total protein and lactate dehydrogenase measured in the *l*th animal; μ is the overall mean for each character; B_i is the fixed effect of *i*th herbal supplement administered; and e_{ijl} is the random residual effect.

Data obtained from the study were subjected to general linear model procedure of analysis of variance using statistical analysis software (SAS, 2003) at $p = 0.05$. Mean differences were separated by the Duncan's multiple range test of the same software.

Results

Proximate and phytochemical analysis of the leaf meals is shown in Table 2. Moringa possess higher crude protein, saponins, glycosides, steroids among the three leaf meals. Mistletoe possess higher crude fiber, ash, nitrogen free extract, alkaloids, flavonoids and tannins among the three leaf meals. Phyllanthus possesses least crude fiber, ash, saponin and tannin among the three leaf meals.

Oxidative status of left and right testis

The biochemical and oxidative status of the left and right testis in bucks fed on feed additives is presented in Table 3. The total protein concentration in the left testis was significantly ($p < 0.05$) higher in bucks fed on basal diet and similar to those of bucks ($p > 0.05$) fed on mistletoe-inclusive diets compared to bucks of other treatments. Higher ($p < 0.05$) values were recorded in the right testis of bucks fed on basal diet and phyllanthus-inclusive diet compared to bucks of other treatments. However, in the left and right testes, the lactate dehydrogenase concentration was significantly ($p < 0.05$) higher in bucks fed on basal diet compared to bucks placed on other treatments. The total antioxidant activity (TAA) in the left testis was significantly ($p < 0.05$) lower in bucks fed on basal diets when compared to bucks of other treatments. However, in the right testis, TAA was significantly ($p < 0.05$) higher in bucks fed on mistletoe-inclusive diets and similar ($p > 0.05$) to those of bucks fed on basal diets compared to bucks of other treatments.

Higher ($p < 0.05$) values of lipid peroxidation were recorded in the left testis of bucks fed on phyllanthus-inclusive diet and similar ($p > 0.05$) to those of bucks fed on moringa-inclusive diet compared to bucks administered with other treatments. Although, in the right testis, higher ($p < 0.05$) values of lipid peroxidation were observed in bucks fed on moringa-inclusive diet compared to bucks of other treatments. The catalase concentration in the left testis was significantly ($p < 0.05$) lower in bucks fed on mistletoe-inclusive diet compared to bucks of other treatments whereas, significantly ($p < 0.05$) higher value of catalase was recorded in the right testis of bucks fed basal diet compared to bucks of other treatments. Higher ($p < 0.05$) values of

Table 2 Proximate and phytochemical analysis of the leafmeals

	Mistletoe leaf meal	Moringa leaf meal	Phyllanthus leaf meal
Dry matter %	89.82	90.76	90.50
Crude protein%	18.81	31.06	27.13
Ether extract %	2.10	2.7	2.5
Crude fiber %	12.10	11.30	9.90
Ash (%)	14.90	12.40	12.06
Nitrogen free extract (%)	52.09	42.54	48.41
Alkaloids (mg/100 g)	14.68	8.5	10.34
Saponins (mg/100 g)	31.01	39.81	23.96
Glycosides (mg/100 g)	75.08	95.92	81.66
Steroids (mg/100 g)	18.82	25.00	20.54
Flavonoids (mg/100 g)	62.93	25.03	44.86
Tannins (mg/100 g)	114.81	96.53	95.98

Table 3 Left and right testicular biochemical and oxidative status of rabbit bucks fed medicinal plants

Parameter	Testis	Basal diet	Moringa-inclusive diet	Mistletoe-inclusive diet	Phyllanthus-inclusive diet	SEM
Total protein (g/L)	L	48.25^{a}	25.17^{c}	43.52^{ab}	34.56^{b}	4.60
	R	43.13^{a}	28.61^{b}	21.90^{b}	55.84^{a}	9.45
Lactate dehydrogenase (U/l)	L	544.61^{a}	101.12^{b}	76.38^{b}	22.59^{c}	95.82
	R	331.61^{a}	38.73^{b}	22.15^{b}	27.43^{b}	48.88
Total antioxidant activity (mmol/liter)	L	0.67^{b}	1.56^{a}	1.58^{a}	1.13^{a}	0.24
	R	1.50^{ab}	1.35^{b}	1.69^{a}	1.06^{b}	0.15
Lipid peroxidation (10^{-3} MDA/mg protein)	L	0.10^{b}	0.19^{ab}	0.08^{b}	0.26^{a}	0.027
	R	0.17^{b}	0.50^{a}	0.18^{b}	0.13^{b}	0.07
Catalase (nmH_2O_2/min/mg protein)	L	24.37^{a}	26.50^{a}	15.80^{b}	22.32^{a}	5.42
	R	53.37^{a}	19.30^{c}	33.32^{b}	29.59^{b}	9.41
Glutathione peroxidase (μgGSH/min/mg protein)	L	50.41^{a}	26.51^{b}	32.75^{b}	22.53^{b}	5.74
	R	48.55^{a}	38.74^{a}	41.78^{a}	27.40^{b}	6.68
Superoxide dismutase (U/min/mg protein)	L	6.63^{a}	1.98^{c}	3.78^{b}	4.50^{ab}	0.79
	R	5.52^{a}	3.25^{b}	2.34^{b}	4.07^{a}	0.78

Different superscript on the same row means significant difference at $p < 0.05$

L, Left; R, Right

glutathione peroxidase were recorded in the left testis of bucks fed on basal diet compared to bucks administered with other treatments.

However, significantly ($p < 0.05$) lower value of glutathione peroxidase was observed in the right testis of bucks fed on phyllanthus-inclusive diet compared to bucks administered with other treatments. The superoxide dismutase level in the left testis was significantly ($p < 0.05$) higher in bucks fed on basal diet and similar to those of bucks ($p > 0.05$) fed on phyllanthus-inclusive diets compared to bucks administered with other treatments. In the right testis, significantly ($p < 0.05$) higher level of superoxide dismutase was recorded in bucks fed on basal diet and those fed on phyllanthus-inclusive diets compared to bucks administered with other treatments.

Oxidative status of the optic lobe, pineal and hypothalamus

The oxidative status of the optic lobe, pineal and hypothalamus in rabbits fed on medicinal plants is depicted in Table 4. The total protein concentration of the optic lobe was significantly ($p < 0.05$) higher in the bucks fed on phyllanthus-inclusive diet compared to bucks of other groups, while significantly ($p < 0.05$) higher values were recorded in the pineal of bucks fed on basal diet and mistletoe-inclusive diets compared to bucks of other groups. However, significantly ($p < 0.05$) higher values were observed in the hypothalamus of bucks fed on mistletoe-inclusive diets, when compared to bucks fed on either basal diet or phyllanthus-inclusive diets. The lactate dehydrogenase level of the optic lobe was significantly ($p < 0.05$) higher in bucks fed on either basal diet or moringa–inclusive diets, when compared to bucks fed on mistletoe-inclusive diet.

More so, significantly ($p < 0.05$) higher value of lactate dehydrogenase was recorded in the pineal of bucks fed on moringa-inclusive diets when compared to bucks of other groups. Whereas, significantly ($p < 0.05$) lower value was recorded in the hypothalamus of bucks fed on phyllanthus-inclusive diet. The total antioxidant activity was significantly ($p < 0.05$) lower in the optic lobe and pineal of bucks fed on either mistletoe or moringa-inclusive diets, respectively, but was significantly ($p < 0.05$) higher in the hypothalamus of bucks fed on either basal diet or phyllanthus-inclusive diets. The optic lobe's lipid peroxidation was significantly ($p < 0.05$) lower in the bucks fed either on basal diet or phyllanthus-inclusive diets. Bucks fed on mistletoe-inclusive diet had significantly ($p < 0.05$)

Table 4 Oxidative status of optic lobe, pineal and hypothalamus in rabbit fed medicinal plants

Parameter	Tissue	Basal diet	Moringa-inclusive diet	Mistletoe-inclusive diet	Phyllanthus-inclusive diet	SEM
Total protein (g/L)	O	26.59^{b}	25.32^{b}	13.89^{b}	39.55^{a}	3.02
	P	24.42^{a}	13.26^{b}	27.97^{a}	17.53^{b}	2.64
	H	14.03^{b}	21.95^{ab}	27.40^{a}	13.25^{b}	2.08
Lactate dehydrogenase (U/l)	O	476.03^{a}	450.75^{a}	300.14^{b}	388.09^{ab}	62.57
	P	298.53^{b}	147.38^{c}	335.64^{b}	416.33^{a}	32.86
	H	228.33^{b}	161.37^{c}	370.07^{a}	61.32^{d}	59.15
Total antioxidant activity (mmol/liter)	O	1.71^{a}	1.44^{a}	0.50^{b}	1.38^{a}	0.23
	P	1.83^{a}	0.54^{b}	1.75^{a}	1.38^{a}	0.16
	H	1.46^{a}	0.71^{b}	0.83^{b}	1.88^{a}	0.19
Lipid peroxidation (10^{-3} MDA/mg protein)	O	0.17^{c}	0.43^{b}	0.70^{a}	0.13^{c}	0.14
	P	1.23^{d}	4.94^{c}	37.28^{a}	9.76^{b}	5.16
	H	0.28^{a}	0.22^{a}	0.16^{b}	0.14^{b}	0.05
Catalase (nmH_2O_2/min/mg protein)	O	34.61^{b}	53.41^{b}	167.38^{a}	35.62^{b}	30.23
	P	4.91^{a}	2.17^{b}	1.78^{b}	0.55^{c}	1.07
	H	182.60^{a}	37.08^{b}	16.42^{b}	11.74^{b}	48.50
Glutathione peroxidase (μgGSH/min/mg protein)	O	85.61^{a}	75.92^{a}	41.23^{b}	77.51^{a}	11.17
	P	2.12^{ab}	1.97^{b}	3.01^{a}	2.66^{a}	0.38
	H	23.58^{b}	42.35^{a}	37.64^{a}	13.81^{c}	5.59
Superoxide dismutase (U/min/mg protein)	O	6.62^{a}	4.37^{b}	2.87^{c}	2.98^{c}	0.86
	P	260.01^{a}	87.73^{b}	148.65^{a}	132.54^{b}	43.21
	H	1.99^{b}	2.56^{b}	4.47^{a}	1.97^{b}	0.27

Different superscript on the same row means significant difference at $p < 0.05$

O, Optic lobe; P, Pineal; H, Hypothalamus

higher pineal lobe lipid peroxidation when compared to bucks of other groups. However, significantly ($p < 0.05$) lower values were recorded in the hypothalamus of bucks fed either on mistletoe or phyllanthus-inclusive diets. The catalase activities were significantly ($p < 0.05$) higher in the optic lobe of bucks fed on mistletoe-inclusive diets when compared to bucks administered with other treatments while bucks fed on basal diet had significantly ($p < 0.05$) higher activities of catalase in the pineal and hypothalamus. Furthermore, significantly ($p < 0.05$) lower concentration of glutathione peroxidase was recorded in the optic lobe of bucks fed on moringa-inclusive diet when compared to bucks of other treatments. In the pineal of bucks fed on mistletoe and phyllanthus-inclusive diets, the glutathione peroxidase concentration was significantly ($p < 0.05$) higher and compared favorably to those fed on basal diet and was better than those of bucks fed on moringa-inclusive diet. The hypothalamic concentration of glutathione peroxidase was significantly ($p < 0.05$) lower in bucks fed on phyllanthus-inclusive diet when compared to bucks administered with other treatments.

The superoxide dismutase concentration in the optic lobe of bucks was significantly ($p < 0.05$) higher than those fed on basal diet when compared to bucks of administered with other treatments while bucks fed on moringa-inclusive diet had significantly ($p < 0.05$) lower concentration of superoxide dismutase in the pineal when compared to bucks administered with other treatments. However, the hypothalamic concentration of superoxide dismutase was significantly ($p < 0.05$) higher in bucks fed on mistletoe-inclusive diet when compared to bucks administered with other treatments.

Oxidative status of the cerebrum, olfactory lobe and cerebellum

The oxidative status of the cerebrum, olfactory lobe and cerebellum of bucks fed on medicinal plants is presented in Table 5. The total protein concentration of the cerebrum was not significantly ($p > 0.05$) affected by the medicinal plants fed to the bucks but was significantly ($p < 0.05$) lower in the olfactory lobe of bucks fed on basal diet. However, significantly ($p < 0.05$) higher total protein concentration was recorded in the cerebellum of bucks fed on mistletoe-inclusive diet and similar ($p > 0.05$) to those of bucks fed on basal diet and moringa-inclusive diets compared to that of bucks fed on phyllanthus-inclusive diet. The lactate dehydrogenase

Table 5 Oxidative status of cerebrum, olfactory and cerebellum in rabbit fed medicinal plants

Parameter	Tissue	Basal diet	Moringa-inclusive diet	Mistletoe-inclusive diet	Phyllanthus-inclusive diet	SEM
Total protein (g/L)	C	22.34	24.74	26.10	22.86	9.69
	OL	16.10^{c}	21.95^{b}	33.64^{a}	26.23^{ab}	3.41
	CB	20.84^{ab}	22.36^{ab}	24.16^{a}	16.49^{b}	1.12
Lactate dehydrogenase (U/l)	C	125.87^{c}	249.58^{b}	111.88^{c}	344.52^{a}	60.25
	OL	254.96^{c}	343.17^{a}	309.82^{b}	250.93^{c}	16.28
	CB	139.58^{b}	258.19^{a}	118.29^{b}	196.87^{a}	53.75
Total antioxidant activity (mmol/liter)	C	1.33^{a}	1.38^{a}	0.33^{b}	1.21^{a}	0.22
	OL	1.04^{ab}	1.08^{ab}	1.54^{a}	0.83^{b}	0.25
	CB	1.59^{b}	1.65^{b}	2.00^{a}	1.38^{c}	0.13
Lipid peroxidation (10^{-3} MDA/mg protein)	C	0.03^{b}	0.07^{a}	0.05^{ab}	0.02^{b}	0.01
	OL	20.20^{a}	11.62^{b}	15.62^{b}	7.48^{c}	3.36
	CB	0.18^{b}	0.34^{a}	0.22^{b}	0.15^{b}	0.05
Catalase (nmH_2O_2/min/mg protein)	C	296.35^{a}	5.57^{b}	5.81^{b}	10.53^{b}	8.55
	OL	3.39^{b}	16.69^{a}	2.92^{b}	1.74^{b}	3.39
	CB	10.74^{c}	55.85^{b}	168.74^{a}	31.15^{b}	13.61
Glutathione peroxidase (μgGSH/min/mg protein)	C	7.79^{b}	9.09^{b}	14.81^{a}	8.12^{b}	1.85
	OL	2.60^{a}	1.30^{b}	3.08^{a}	1.47^{b}	0.37
	CB	30.68^{a}	29.77^{a}	22.46^{b}	19.74^{b}	3.66
Superoxide dismutase (U/min/mg protein)	C	0.76^{b}	0.64^{b}	0.97^{a}	0.87^{a}	0.07
	OL	52.07^{b}	31.38^{b}	52.91^{b}	189.08^{a}	40.84
	CB	3.65^{a}	3.43^{a}	1.49^{c}	2.41^{b}	0.49

Different superscript on the same row means significant difference at $p < 0.05$

C, Cerebrum; OL, Olfactory; CB, Cerebellum

levels of the cerebrum and olfactory lobes were significantly ($p < 0.05$) higher in bucks fed on phyllanthus-inclusive diets and moringa-inclusive diets, respectively, when compared to bucks administered with other treatments. More so, significantly ($p < 0.05$) higher values of lactate dehydrogenase were recorded in the cerebellum of bucks fed on either moringa or phyllanthus-inclusive diets when compared to bucks of other groups.

The total antioxidant activity was significantly ($p < 0.05$) lower in the cerebrum of bucks fed on mistletoe-inclusive diets while significantly ($p < 0.05$) higher TAA was recorded in the olfactory lobes of bucks fed on mistletoe-inclusive diet, but similar ($p > 0.05$) to those fed on either basal diet or moringa–inclusive diets but better than bucks fed on phyllanthus-inclusive diet. Furthermore, the cerebellum's TAA was significantly ($p < 0.05$) higher in bucks fed on mistletoe-inclusive diet. The cerebrum's lipid peroxidation was significantly ($p < 0.05$) higher in the bucks fed on moringa-inclusive and similar ($p > 0.05$) to those fed on mistletoe-inclusive diets but better than bucks fed on either basal diet or phyllanthus-inclusive diets. Bucks fed on either basal diet or moringa-inclusive diet had significantly ($p < 0.05$) higher olfactory lobe and cerebellum lipid peroxidation, respectively, when compared to bucks of other groups. The catalase activity was significantly ($p < 0.05$) higher in the cerebrum of bucks fed on basal diets when compared to bucks administered with other treatments while bucks fed on either moringa or mistletoe-inclusive diets had significantly ($p < 0.05$) higher activities of catalase in the olfactory lobe and cerebellum.

Furthermore, significantly ($p < 0.05$) higher concentration of glutathione peroxidase was recorded in the cerebrum of bucks fed on mistletoe-inclusive diet compared to bucks administered with other treatments. In the olfactory lobes of bucks fed on basal diet and mistletoe-inclusive diets, the glutathione peroxidase concentration was significantly ($p < 0.05$) higher when compared to bucks of other groups. A significantly ($p < 0.05$) higher cerebellum concentration of glutathione peroxidase was recorded in bucks fed on basal diet and moringa-inclusive diets when compared to bucks administered with other treatments. The superoxide dismutase concentration was significantly ($p < 0.05$) higher in the cerebrum of bucks fed on mistletoe and phyllanthus-inclusive diets when compared to bucks administered with other treatments while bucks fed on phyllanthus-inclusive diet had significantly ($p < 0.05$) higher concentration of superoxide dismutase in the olfactory lobe when compared to bucks administered with other treatments. However, a highly ($p < 0.05$) significant cerebella concentration of superoxide dismutase was observed in bucks fed on basal diet and mistletoe-inclusive diets compared to bucks of other treatments.

Discussion

Male infertility ranks among the most debilitating global health challenge with accusing fingers pointing at oxidative stress. This drives the point that attainment of a balance between free radicals' production and antioxidant activities in the testes is crucial (Jimoh and Ewuola 2018).

The trend of result shows that testicular total protein in bucks fed on moringa is lower than those fed on basal diet and phyllanthus. The lower testicular protein of moringa fed bucks could adversely affect spermatogenesis. As previously reported by Ewuola et al. (2014), that reduction in testicular total protein can affect the glycoprotein hormones and can lead to impairment in the function of testis, which may result in decreased spermatozoa production.

Lactate dehydrogenase (LDH) is required for metabolic processes, which provides energy metabolism of sperm cells, catalyses the oxidation of lactate, thereby maintaining sperm motility, survival and fertility of spermatozoa (Viudes-de-Castro et al., 2015; Sopkova et al., 2015). Saleh et al. (2015) reported that testicular LDH is localized in mitochondria of primary spermatocytes and it is associated with the maturation of germinal epithelial layer of seminiferous tubules and could account for its requirement in ATP phosphorylation. In the current study, the inclusion of phytogenic additives in the diet of bucks reduced testicular LDH compared to those fed on basal diet. This shows that the feed additives have negative effect on the metabolic activity of the testis and would adversely affect spermatozoa kinetics. It could also be due to oxidative stress as reported by Sawiress et al. (2011) and Saleh et al. (2015) that rats under oxidative stress had reduced testicular lactate dehydrogenase activity. Similarly, Saleh et al. (2015) reported that LDH correlates with the motility and liveability of sperm cells, and thus lower activity of LDH in the testis of phytogenic fed bucks could lead to lower sperm quality. Contrariwise, Alya and Azhar (2013) reported higher amounts of LDH in the testis of newly born rats and where its activity reduces with the development of the testis with age indicates that LDH are associated with the maturation of the germinal epithelial layer of seminiferous tubules. Thus, higher activity of testicular LDH suggest that deterioration of germinal epithelium in basal diet fed bucks.

Total antioxidant activity of the left testis was enhanced by the inclusion of phytogenics, while total antioxidant activity of right testis in bucks fed on phytogenic additives were similar to bucks on basal diet. This shows that the phytogenic additives confer antioxidant enrichment on testicular tissues and could lead to reduced

spermatozoa apoptosis in testis and improved epididymal spermatozoa maturation (Suresh et al., 2010). Similarly, Collodel et al. (2015) reported that antioxidant substances can give protection from oxidative damage to reproductive tissues and germ cells. This is similar to report of Selim et al. (2021) on moringa supplementation in rabbit enhanced antioxidant defence and reduced peroxides in serum and meat via modulating poly unsaturated fatty acids, resulting from the phenolic compounds, polyphenolic, and flavonoids.

However, testicular lipid peroxidation was lowered in mistletoe fed bucks and compared favorably with the basal diet. Conversely, bucks fed on moringa and phyllanthus had higher testicular lipid peroxidation, which indicates oxidative stress in the bucks' testis, and could account for its lowered testicular protein and/or LDH. Testicular membranes are highly susceptible to oxidative stress due to higher thiobarbituric acid reactive substances (TBARS), with detrimental effects on testicular tissue because of their high content of poly unsaturated fatty acid (PUFA) (Tothova et al., 2013). Similar report by Alya and Azhar (2013) revealed that reduced testosterone level in bucks fed on moringa supplements results in failure to maintain proper spermatogenesis, which is evident in decreased sperm production, and could be linked to oxidative stress in testicular tissue. In contrast, extracts of *Lycium barbarum* protects the testes from oxidative damage and suppress oxidative DNA damage in mouse testicular cells induced by heat stress (Zhang et al., 2013).

The elimination and decomposition of H_2O_2 (produced by anaerobic metabolism of spermatozoa) in the testes is predominating and effected by glutathione peroxidase and catalase, where it is critical for detoxification of peroxidized lipids and metabolism of xenobiotics. Testicular glutathione peroxidase (GPx) was lower in phyllanthus fed bucks compared to those fed the basal diet while testicular GPx in bucks fed on moringa and mistletoe were similar with the basal diet. Similarly, testicular catalase activity was lower in mistletoe fed bucks compared to those on basal diet, while the least values were obtained in the right testis of bucks fed on moringa. Increase in catalase activity is part of the defence system in the testis against oxidative stress (Pasko et al., 2011). Phytogenic additives reduce hydrogen peroxide scavenging ability of testicular cells and could account for oxidative stress in the testicular tissue. Similarly, lower doses of Amaranth seeds decrease catalase and GPX activity of rat testis (Pasko et al., 2011).

Superoxide dismutase functions in association with catalase and glutathione to control superoxide anion and hydrogen peroxide generation and/or accumulation in the management of oxidative stress (Jimoh et al., 2018a). In this study, testicular superoxide dismutase (SOD) of bucks fed phyllanthus and basal diet were better than bucks fed on moringa and mistletoe.

The trend of result shows that the antioxidants enzymes of testis in rabbit fed on phytogenic additives were not better than basal diet, despite high testicular total antioxidant activity. Reduction of antioxidant enzymes activity in testicular tissue might be due to accumulation of free radicals, causing enhanced lipid peroxidation or inactivation of the antioxidant enzymes (Khan, 2012). This suggests that the non-enzymatic antioxidants account for the higher testicular total antioxidant activity and were not as effective in protecting the testicular tissues against lipid peroxidation. This is in line with the position of Aitken and Roman (2008) when they did posit that oxidative stress is a consistent feature of testicular physiology, capable of disrupting the steroidogenic capacities of Leydig cells and the germinal epithelium to differentiate normal spermatozoa.

Total protein of optic lobe, pineal, hypothalamus, olfactory lobe and cerebellum in bucks was highest in phytogenic fed groups (phyllanthus, mistletoe, mistletoe, mistletoe and mistletoe, respectively). The inclusion of the three phytogenic agents did not adversely affect protein synthesis and the rate of turnover in the optic lobe, pineal, hypothalamus, olfactory lobe and cerebellum.

Lactate dehydrogenase of pineal, hypothalamus, cerebrum, olfactory lobe and cerebellum in bucks were highest in phytogenic fed groups (phyllanthus, mistletoe, phyllanthus, moringa, moringa, respectively), while LDH of optic lobe in bucks fed on phytogenics was similar to those on basal diet except mistletoe fed groups. This highlights the contribution of phytogenics to glucose mobilization for brain cells. It is reported that tissues that display intense but short increases in LDH activity generally can maintain intense glycolytic rates and yield high amounts of lactate (Gupta, 2022). As lactate is an important energy metabolite for brain cells, making cellular localization of lactate dehydrogenase important to oxidize lactate to pyruvate and also converts pyruvate to lactate. Thus, LDH is at the interchange between glycolysis and tricarboxylic acid cycle (Gupta, 2022).

The brain is highly sensitive to oxidative stress due to abundance of PUFA, deficiency in antioxidant defence, high rate of oxygen utilization due to higher metabolic rate and the presence of high transition metals like copper and iron in several regions, which could lead to formation of hydroxyl radical (Singh et al., 2019).

This study reveals that total antioxidant activity of cerebellum in bucks on mistletoe was higher than other treatments, with the least recorded in bucks on phyllanthus. While total antioxidant activity of cerebrum, optic lobe, pineal and hypothalamus in bucks on phytogenics were similar to basal diet except phyllanthus, mistletoe,

moringa and both mistletoe and moringa, respectively, that were lower. Cerebellum is responsible for integrating sensory and motor functions and coordinates the brain's other processes including neurosecretions (Zhang et al., 2023), thus maintaining oxidative stability in the tissues is imperative for body coordination. This supports Pasko et al. (2011), which reported that consumption of plants rich in antioxidants compounds improve activities of free radical scavenging enzymes in tissues during oxidative stress, and agrees with in vitro studies, which indicated that dietary antioxidants can protect against oxidative damage in some tissues (Farombi and Owoeye, 2011). The antioxidant mechanism of herbs includes scavenging free radicals, interacting with oxidative cascade and preventing its results, oxygen quenching and making it less available for oxidative reaction, inhibition of oxidative enzymes, chelating and disarming oxidative properties of metal ions (Kandemir et al., 2011; Jimoh et al., 2018a).

The trend of result shows that lipid peroxidation of optic lobe, pineal, hypothalamus, cerebrum, olfactory lobe and cerebellum were lowest in bucks on basal diet, basal diet, mistletoe, phyllanthus, phyllanthus and phyllanthus, respectively, while the highest values were observed on phytogenics fed bucks except olfactory lobe were bucks on basal diet had highest values. This indicates that some organs have stronger capacity than blood or other organs to reduce lipid peroxidation, as reported by Pasko et al. (2011). And this could be due to the difference in ROS surge in different brain regions, owing to heterogeneity and metabolic compartmentalization of the brain mitochondria (Basha et al., 2010).

In the current study, low lipid peroxidation was observed in cerebellum of bucks with the inclusion of phyllanthus. Cerebellum has been reported to be susceptible to oxidative stress following exposure of rats to BDE-99, which caused difficulty in the inverted screen task (Bellés et al., 2010). Consequently, the phyllanthus inclusion can be used to tackle peroxides accumulation in cerebellum. Also, in the current study, high lipid peroxidation was observed in olfactory lobe and pineal compared to other brain tissues assessed. Inclusion of the phytogenic additive increased lipid peroxidation in pineal, while its inclusion reduced lipid peroxidation in olfactory lobe.

Catalase removes H_2O_2 faster and is physiologically operative at higher concentrations of hydrogen peroxide, while glutathione peroxidase is efficient at lower cellular H_2O_2 levels, but it also metabolizes lipid peroxides (Ransy et al., 2020). High catalase activity of optic lobe, pineal, hypothalamus, cerebrum, olfactory lobe and cerebellum were observed in bucks fed on mistletoe, basal diet, basal diet, basal diet, moringa and mistletoe, respectively. Reduction in catalase activity in the cerebellum has been previously reported (Castro et al., 2021) reflects an inability of cerebellum to scavenge hydrogen peroxide, or by enzyme activation due to excess reactive oxygen species (ROS) production in the tissue. This could be combated by the administration of phytogenic additives as obtained in this study, which shows that bucks fed on the three phytogenic additives had higher catalase activity the basal diet.

The brain's low content of glutathione increases its vulnerability to free radicals, the high proportion of PUFAs in its membranes and its metabolism of about 20% of total body oxygen (Benyettou et al., 2017). However, this study shows that GPx activity in hypothalamus, cerebrum, olfactory lobe and pineal was enhanced in bucks fed on moringa and mistletoe when compared to bucks administered with other treatments while the GPx activity in the optic lobe and cerebellum in groups fed with phytogenics compared favorably with the basal diet.

Thus, administration of moringa and mistletoe as feed additives could ameliorate decrease of total glutathione that disturb the activities of antioxidant enzymes in the brain of rats as reported by Benyettou et al. (2017). Previous reports suggest that the activity of enzymatic and non-enzymatic antioxidants is not uniform in brain regions, with the medulla and hippocampus appearing to be more vulnerable due to low levels of glutathione compared to cerebrum and cerebellum (Basha et al., 2010).

Elevation of catalase and glutathione peroxidase activities suggest that the concentration of hydrogen peroxide is increased above a tolerable ROS level, and this necessitates its faster elimination (Mitrović et al., 2018). When glutathione peroxidase is elevated along with catalase, it is an indication that the ROS attack involved lipid molecules as well (Mitrović et al., 2018).

The phytogenic additive fed bucks compared favorably with the basal diet in respect of SOD in cerebellum and pineal. Similar to Zhang et al. (2022) who showed that SOD deficiency exacerbated cerebral infarction, while chronic administration of omega-3 over 6 weeks increased SOD activity and elevated reduced glutathione, leading to effective reduction of the brain lipid peroxides. An inadequate free radical/reactive oxygen species sequestration is a consequence of decreased antioxidant enzymes activities, which may ultimately lead to oxidative assault of all class of biomolecules (Djuric et al., 2015). Similarly, rise in lipid peroxidation was attributed to an inhibition of SOD in the rat brain as a result of substantial rise in phospholipid peroxidation in brain cells, causing membrane damage and neuron death (Benyettou et al., 2017).

Following severe ischemic insult; antioxidant supplements could mitigate some of the symptoms linked with cerebral injury by enhancing antioxidant capacity,

reducing lipid peroxidation, inducing chaperon molecules and stabilizing membrane integrity (Briones-Valdivieso et al., 2024). Thus, phytogenic additives are potent in reducing lipid peroxidation in brain regions and enhancing antioxidant activity, which can ameliorate cerebral injury. This study is in agreement with the thought that herbs and its various phytoconstituents such as phenolic acids and flavonoids could change antioxidative status in tissues (Pasko et al., 2011). Also, reports had indicated that herbal supplements such as moringa possess polyphenolic compounds which are capable of inducing a variety of biological properties and are excellent source of essential nutrients to present a valuable feed ingredient for promoting the physiology of growing rabbits (Selim et al., 2021), Broilers (Saleh et al., 2018) and laying hens (Saleh et al., 2019).

The interplay between these phytochemicals and their oxidative effects indicates a complex mechanistic integration: the antioxidants from these supplements likely scavenge reactive oxygen species (ROS), reducing oxidative stress in both brain and gonadal tissues. This reduction in ROS can protect cellular integrity and function, supporting spermatogenesis in the gonads and maintaining cognitive and hormonal functions in the brain. Moringa, with its high crude protein, saponins, glycosides, and steroids, shows a capacity to enhance protein synthesis and lactate dehydrogenase activity in the testis, essential for spermatogenesis and energy metabolism. However, the increase in lipid peroxidation indicates a potential for oxidative damage, which could impair testicular function if not counterbalanced by sufficient antioxidants.

Mistletoe, rich in crude fiber, ash, and potent antioxidants like flavonoids and tannins, significantly boosts total antioxidant activity in various brain regions (optic lobe, pineal, hypothalamus) and reduces lipid peroxidation. This enhanced antioxidant defense can protect neural tissues from oxidative stress, supporting cognitive functions and hormonal regulation via the pineal and hypothalamus. The improvement in oxidative status in the brain might also influence the hypothalamic-pituitary–gonadal axis, optimizing reproductive functions.

Phyllanthus, despite its lower crude fiber and ash content, effectively modulates oxidative balance, particularly by enhancing superoxide dismutase levels in the brain and reducing oxidative damage. This suggests a role in maintaining cellular redox homeostasis, which is crucial for both brain function and spermatogenesis.

Conclusion

The study demonstrates that different leaf meals have distinct impacts on the nutritional and oxidative status in various tissues of rabbit bucks. Moringa, rich in crude protein, saponins, glycosides, and steroids, enhances protein and lactate dehydrogenase levels but increases lipid peroxidation in the testis. Mistletoe, with high crude fiber, ash, and antioxidants like flavonoids and tannins, boosts total antioxidant activity in several brain regions and reduces lipid peroxidation, indicating its potential for reducing oxidative stress. Phyllanthus, having the least fiber and ash but effective antioxidant properties, notably affects the oxidative balance in both the testis and brain, with varied impacts on different tissues. These findings suggest that selecting specific leaf meals can strategically modulate oxidative stress and improve the overall health and reproductive performance of bucks, emphasizing the need for tailored dietary interventions in animal husbandry.

Abbreviations

TAA	Total antioxidant activity
LDH	Lactate dehydrogenase
ATP	Adenosine triphosphate
TBARS	Thiobarbituric acid reactive substances
PUFA	Poly unsaturated fatty acid
GPx	Glutathione peroxidase
SOD	Superoxide dismutase
ROS	Reactive oxygen species

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Author contributions

Jimoh O.A. designed the study, monitored the experimental protocol, supervised the study and manage the sample collection, wrote the final manuscript.

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Availability of data and materials

Available on request.

Declarations

Ethical approval and consent to participate

The institutional ethics committee approved the research for care and use of animal for research (approval no: FPA/EC/20/0405). Institutional and national standards for the care and use of animals for research were followed, to minimize pain on the animals.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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