

2 **Combined oral arginine and monosodium**
3 **glutamate exposure induces adverse response**
4 **on the prostate **function** and testis **histology** of**
5 **rats**

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15 **ABSTRACT**

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Aims: This study investigated the effect of exposure to arginine (ARG) and glutamate (GLU), or its variant, monosodium glutamate (MSG) on the prostate function and testis histology of rats.

Study design: Exposure to either ARG, GLU, monosodium glutamate (MSG), ARG plus GLU or ARG plus MSG was per oral for 4 consecutive weeks. On the last day of the experiment, rats were food-deprived for 15 h before collecting their blood and testis samples.

Place and Duration of Study: Department of Biochemistry and Department of Veterinary Pathology, University of Nigeria Nsukka, Nigeria, between June 2005 and June 2006.

Methodology: Total and prostatic acid phosphatase activities in serum were determined by the method of Walter and Schutt. Testis sections were stained and mounted using haematoxylin and eosin (H&E), for histology.

Results: On comparison with control, the results showed that ARG, GLU, or arginine together with monosodium glutamate (ARG+MSG) induced a significant ($p < 0.05$ and $p < 0.01$) elevation whereas, ARG+GLU caused a reduction ($p < 0.05$ and $p < 0.01$), in serum total acid phosphatase (TAP) and prostatic acid phosphatase (PAP) activities. MSG-induced reduction in TAP activity (20.76 ± 0.18 I.U/L), however, was not statistically significant ($p > 0.05$ and $p > 0.01$). Histological examination of the testis sections revealed varying degree of degeneration characterized by necrosis in ARG+GLU and ARG+MSG groups relative to control and ARG, GLU or MSG groups.

Conclusion: Results may indicate variable treatment related adverse effect on the prostate function and the testis histology of the rats. The possible effect, however, appeared higher following concomitant exposure to ARG and MSG. Thus, caution should be exercised in the simultaneous ingestion of arginine and monosodium glutamate in animals. **Further work however, is required to address some shortcomings (including small sample size) of this study.**

17
18 **Keywords:** *Testis histomorphology. Arginine. Glutamate. Prostatic acid phosphatase.*
19 *Prostate.*

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22 1. INTRODUCTION

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24 Prostate dysfunction related diseases, notably prostate cancer is on the increase worldwide.
25 Conceivably, testis dysfunction may be linked with prostate pathologies since the conversion
26 of testosterone secreted by the Leydig cells in testis [1] into its more active form,
27 dihydrotestosterone (DHT), occurs in the prostate [2]. This conversion stimulates the
28 proliferation of prostate cells resulting in prostate enlargement or benign prostate
29 hyperplasia [3].

30 The amino acids arginine and glutamate exert important physiological functions probably
31 due to their unique roles in the synthesis of important bioactive substances, notably nitric
32 oxide. In particular, L-arginine (ARG) plays multiple physiological functions in animals [4,5,6].
33 These include, attenuation of the stress response [7,8,9], immune function enhancement
34 [10,11], protein synthesis regulation [12]), and promotion of wound healing [13]. However, it
35 was shown that arginine mediated these physiological functions via its important
36 metabolites, notably nitric oxide [14,15,16,17]. Thus, the unique role of glutamate (GLU) in
37 activating nitric oxide synthase enzyme (via calcium-calmodulin complex formation) [18])
38 suggests that it may enhance ARG-induced effect related to nitric oxide synthesis. This may
39 explain the increasing use of L-arginine and glutamate in diets and drugs.

40 However, despite the promising benefits, a number of studies have shown that these amino
41 acids (arginine and glutamate) may elicit adverse effect in animals. For instance, reports
42 showed that monosodium glutamate (MSG), a variant of GLU, induced adverse effects in
43 experimental animal models [19,20,21,22,23]. Furthermore, reports implicated ARG for
44 increasing systemic blood pressure (at 4 mg ml⁻¹ in drinking water) in rats [24], and other
45 pathological conditions via excessive production of nitric oxide, its major metabolite [25].

46 Thus, this study assessed the potential effect of exposure to ARG (60 mg/kg bw), GLU (90
47 mg/kg bw), MSG (15 mg/kg bw), ARG+GLU (60:90 mg/kg bw) or ARG+MSG (60:15 mg/kg
48 bw) on the functionality of the prostate gland (using serum total and prostatic acid
49 phosphatase) and the testis (using histological examination) in male rats. Acid phosphatase
50 activity was elevated in the sera of males with metastatic prostatic cancer [26,27,28],
51 indicating that increased serum acid phosphatase levels is of great clinical importance in the
52 diagnosis of prostate dysfunction. The choice of treatment dose was based on the earlier
53 reports [29,30] and WHO reported daily oral intake of these test agents [31].

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56 2. METHODOLOGY

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58 2.1. Animals and treatment

59 A total of 24 Wistar albino rats (male, from different litter) were used in this experiment. Their
60 approximate weight and age (66 g, 7 weeks of age) were similar to those used by Amin and
61 Nagy [32]. All rats were housed in the animal facility of Home Science and Nutrition
62 Department, University of Nigeria Nsukka, Nigeria. After a week acclimatization, they were
63 allotted randomly to one of the six oral exposures based on body weight in a completely
64 randomized design. Each exposure consisted of four rats, just enough to obtain valid and
65 meaningful results [33]. Rats in Group 1 (the control) were intubated distilled water (3 ml/kg
66 bw, corresponding to the volume used in dissolving the various test agents). On the other
67 hand, rats in Groups 2, 3, 4, 5 and 6 were intubated ARG (60 mg/kg), GLU (90 mg/kg), MSG
68 (15 mg/kg), ARG+GLU (60:90 mg/kg) and ARG+MSG (60:15 mg/kg), respectively. The
69 doses were calculated and adjusted based on the WHO recommended daily oral intake of

70 these agents for an average person of 70 kg. Exposure was per oral and lasted for 28
71 consecutive days. MSG (>98% purity) was purchased from a regular foodstuff market at
72 Nsukka. ARG and GLU (>98% purity) were obtained from the chemical store of Biochemistry
73 Department, University of Nigeria, Nsukka.

74 **2.2. Sample collection and preparation**

75 At the end of experiment, 15 h after the last feeding, the rats were sacrificed to obtain blood
76 samples at 8:00 a.m, by retro orbital sinus venipuncture using sterile capillary tubes
77 (containing no anticoagulant) as described by Egbuonu et al. [34], followed immediately by
78 excision to obtain the testis samples. This study was carried out in accordance with ethical
79 guidelines for animal welfare and approved by Biochemistry Department, Faculty of
80 Biological Sciences, University of Nigeria Nsukka, Nigeria.
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82 Blood samples were centrifuged for 10 min at 3,000g, room temperature, and the serum was
83 stored in deep freezer for assays of biochemical parameters. Following excision, testis
84 samples were collected immediately and fixed in 10% formaldehyde buffered saline (formal
85 saline) for histological examination. The testis sections were stained and mounted using
86 haematoxylin and eosin (H&E), as described earlier [35]. In brief, the testis specimens were
87 dehydrated in graded levels of alcohol (70-100%) in ascending order to remove the water
88 content. After dehydration, the tissues were cleared in xylene impregnated with paraffin wax
89 and sectioned at 5 microns thickness using rotary microtome. The sections were floated on a
90 water bath maintained at a temperature of 2-3°C below the melting point of the paraffin wax
91 after which the sections were dried on a hot plate maintained at a temperature of 2-3°C
92 above the melting point of the paraffin wax. After drying, the sections were stained and
93 mounted using haematoxylin and eosin.

94 **2.3. Assay of the serum total and prostatic acid phosphatase activities**

95 Total and prostatic acid phosphatase in serum were determined by the method of Walter and
96 Schutt [36] based upon the principle that acid phosphatase reacts with p-nitrophenyl
97 phosphate in alkaline medium to produce colored p-nitrophenol that is measured with a
98 spectrophotometer (NOVASPEC LKB Biochrome, model 4049, Germany) at 405 nm.
99 Thereafter, the activity of the serum PAP was obtained by the difference between the
100 sample and the blank absorbance readings.

101 **2.4. Statistical analysis**

102 Values are expressed as mean \pm SD or SEM. Data were analyzed by one-way analysis of
103 variance (ANOVA) and Bonferroni post hoc (multiple comparisons) test. All statistical
104 analyses were performed using Statistical Package for Social Sciences (SPSS version 16;
105 SPSS Inc., Chicago, IL., USA). Differences were considered significant at $p < 0.05$ and
106 $p < 0.01$ levels of significance. Results were correlated for association using Pearson's and
107 Spearman's rho bivariate or two-tailed (r (p) = 0.05 and r (p) = 0.01) methods.

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3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Serum prostatic acid phosphatase (PAP) activity

116 The effect of the various exposures on the serum prostatic acid phosphatase of rats are
117 summarized in Table 1. Contrary to control, exposure to ARG (Group 2) or GLU (Group 3)
118 significantly ($p < 0.05$ and $p < 0.01$) increased serum PAP activity in rats whereas exposure to
119 MSG (Group 4) reduced ($p < 0.05$ and $p < 0.01$) the same parameter in rats. Concomitant
120 exposure to ARG and GLU (Group 5), however, decreased ($p < 0.05$ and $p < 0.01$) serum PAP
121 activity in rats when compared with control or other exposure groups whereas the same
122 parameter increased ($p < 0.05$ and $p < 0.01$) above the other treatment groups in rats exposed
123 to ARG together with MSG (Group 6).

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3.1.2. Serum total acid phosphatase (TAP) activity

126 As shown in Table 1, TAP activity in serum increased ($p < 0.05$ and $p < 0.01$) in rats
127 concomitantly exposed to ARG and MSG (Group 6). It also increased ($p < 0.05$ and $p < 0.01$) in
128 the rats exposed to only ARG (Group 2) or GLU (Group 3). On the other hand, TAP activity
129 in serum decreased ($p > 0.05$ and $p > 0.01$) in rats exposed to only MSG (Group 4) but
130 decreased ($p < 0.05$ and $p < 0.01$) in those exposed to ARG in combination with MSG (Group
131 6).

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3.1.3. Histomorphological changes in the testis sections

134 The histomorphological changes in the testis sections of the different groups of treated rats
135 were characterized by degenerative/necrotic and inflammatory changes as evidenced by
136 lesions (Figures 2, 3 and 4). Sections of testis from rats in the control (Group 1), showed the
137 normal histological features for the seminiferous tubules and interstices of rats [Figure 1].
138 The tubules showed normal cells of the different stages of spermatogenesis (from
139 spermatogonia to spermatids), Sertoli cells and interstitial (Leydig) cells.

140 Sections collected from rats exposed to ARG (Group 2) showed histological features similar
141 to those of control (Group 1) rats. Exposing rats to GLU (Group 3) produced mild
142 hyperaemia of the testis, with moderate oedema fluid in the interstices [Figure 2]. The
143 oedema fluid was essentially a transudate as it was devoid of inflammatory cells. The
144 different spermatogenic cells were normal in appearance, and there were many spermatids
145 present.

146 The testis of rats treated with MSG (Group 4) showed similar, but more severe
147 histomorphologic changes when compared with Group 3 rats. The oedema fluid in the
148 interstices was an exudate, with lots of inflammatory cells, the spermatids were fewer, and
149 the interstitial (Leydig) cells showed moderate degeneration [Figure 3].

150 Concomitant exposure to ARG+GLU (Group 5) and ARG+MSG (Group 6) increased the
 151 severity of lesions. The population of normal spermatogonia cells reduced severely; with
 152 mild to moderate reduction in the number of Sertoli cells in the basement membrane of the
 153 tubules, and Leydig cells in the interstices of the sections [Figure 4]. Spermatids were very
 154 few to totally absent in the tubules. Generally, treating the rats with ARG, GLU or MSG alone
 155 seem to have had mild effect on the histomorphology sections of the testis, whereas feeding
 156 ARG with GLU or MSG seem to have enhanced the lesions (Histopathologist personal
 157 opinion).

158 3.2. Discussion

159 L-Arginine, the physiological precursor of important bioactive substances, including nitric
 160 oxide, polyamines, creatine, agmatine, glutamate, and proline [37,38], is a notable
 161 constituent of sex enhancing supplements. L-glutamate is a food additive widely used in the
 162 form of its variant, monosodium glutamate, for the flavor enhancing potential. These amino
 163 acids could be present together in diets and drug thus, it is important to determine whether
 164 exposure to arginine, glutamate, or monosodium glutamate either alone or in their possible
 165 combinations could adversely impact on the functional capacity of the prostate and testis
 166 histology of rats.

167 Prior to the establishment of prostate specific antigen (PSA) [39], elevated TAP and PAP
 168 activities were among the main common clinical features of prostate pathologies hence were
 169 used to assess the functional capacity of the prostate [40,41]. In particular, the TAP value for
 170 control in the present study was within the range reported by Uboh et al. [42] and Anosike et
 171 al. [43]. Thus, the rise in the serum TAP and PAP activities [Table 1] noted in ARG, GLU or
 172 ARG+MSG fed rats could be reflective of apparent adverse response on the prostate glands
 173 functionality.

174 **Table 1:** The effect of exposure to DW, ARG, GLU, MSG, ARG+GLU, ARG+MSG, on serum
 175 PAP and TAP activities in rats serum (ANOVA followed by Bonferroni *post hoc* test)

Groups	PAP activity (I.U/L)	TAP activity (I.U/L)
	Mean ± S.D	Mean ± S.D
DW	18.69 ± 0.14	20.85 ± 0.14
ARG	48.58 ± 0.15*	52.67 ± 0.18*
GLU	21.08 ± 0.10*	22.46 ± 0.17*
MSG	7.63 ± 0.18*	20.76 ± 0.18 ^a
ARG+GLU	9.72 ± 0.21*	19.71 ± 0.17*
ARG+MSG	76.53 ± 1.07*	80.17 ± 0.18*

176 Sample number, n = 4; * The mean difference is significant at the 0.01 and 0.05 levels.
 177 ^a The mean difference is significant with other groups but not significant with control at 0.01 and 0.05 levels.
 178 TAP and PAP activities of the various groups correlated positively at r (p)=0.01
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180 This may be so since the elevation of serum level of these bio-markers was associated with
 181 prostatic cancer [26,27,28,44]. Thus, prostate dysfunction possibly induced by exposure to
 182 ARG, GLU or ARG+MSG may have resulted in the increased TAP and PAP levels noted in

183 this study, indicating that exposure to ARG, GLU or ARG+MSG probably predisposed the
184 rats to incident prostatic disorders. However further study, perhaps using PSA and
185 histopathologic examination of the prostate tissue, is required to identify/confirm the specific
186 prostatic disorder risks associated with exposure to ARG, GLU or ARG+MSG.

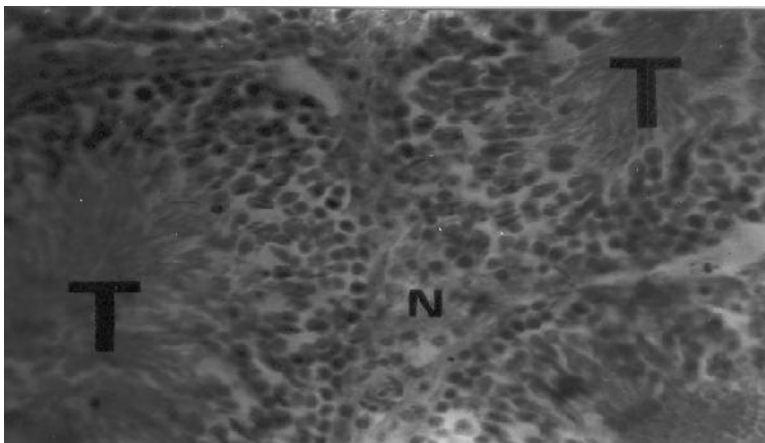
187 On comparison with control rats [Figure 1], variable histomorphological changes were noted
188 in the testis sections of other groups, suggesting variable injury [45] on the testis, possibly
189 related to the various treatments.

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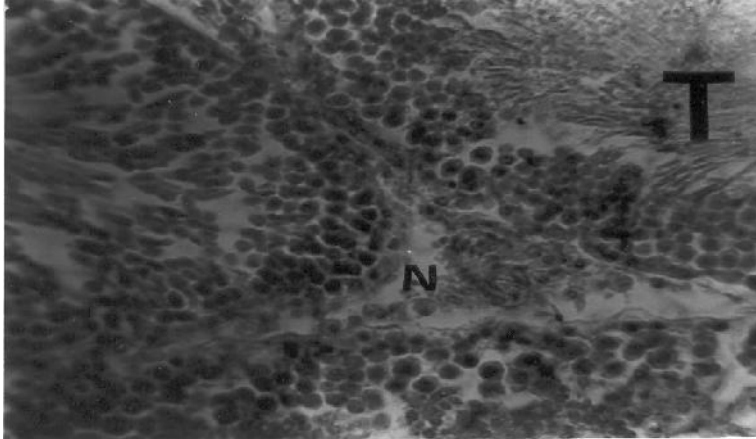
194 **Figure 1:** Section of testis from untreated, control (DW) (Group 1) rat showing seminiferous
195 tubules (T) and interlobular (interstices) spaces (N) showing normal Leydig cells. H & E
196 stains, ×400
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199 In particular, sections collected from rats treated with ARG showed histologic features similar
200 to those of control (DW) rats, indicating non adverse influence on the testis following ARG
201 exposure to rats. Earlier, Fahim et al. [46] reported no significant change in weight and
202 histological structure of testes, epididymides, and seminal vesicles following exposure to
203 ARG, even in combination with zinc.

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205 Exposing rats to GLU may have produced mild hyperaemia of the testis, indicated by
206 moderate oedema fluid in the interstices but devoid of inflammatory cells [Figure 2].

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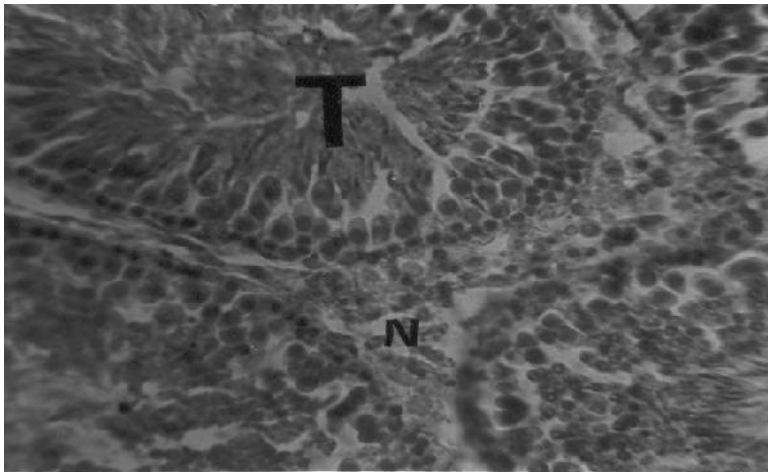


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Figure 2: Sections of testis from rat treated with GLU (Group 3) showing seminiferous tubule (T) with lots of spermatids, and oedematous interstitial space (N). H & E stains, ×400

212 However, the histomorphologic changes noted in the MSG-fed rats [Figure 3] were severe
213 as compared with control or GLU-fed rats. This is consistent with previous work [47],
214 suggesting that exposure to MSG may consequently impair spermatogenesis or testosterone
215 production in the rat models [48].

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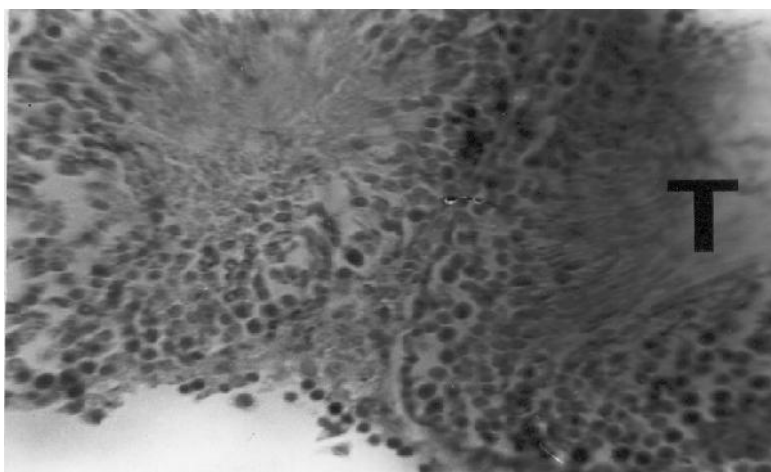
Figure 3: Section of testis from rat treated with MSG (Group 4) showing seminiferous tubule (T) with only few spermatids and interstitial space (N) with inflammatory exudates. H&E stains, ×400

222 We did not explore whether or not the possibly impaired spermatogenesis or testosterone
223 production accounted for the apparent MSG-induced reduction in serum TAP and PAP
224 activities observed in this study. However, conflicting result especially with increasing
225 concentration of MSG was reported [49], hence could be a worthwhile area for further study.

226 Furthermore, treating rats with ARG+GLU or and ARG+MSG increased the severity of
227 lesions [Figure 4], probably highlighting the enhanced adverse influence on the testis of
228 Wistar rats following concomitant exposure to ARG and either GLU or MSG. **Oddly, the**

229 histomorphological changes were inconsistent with the biochemical changes in Group 4
230 (MSG) and Group 5 (ARG+GLU), but histomorphological changes were more definitive
231 response following agent treatment in animals [35].

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235 **Figure 4:** Section of testis from rat treated with ARG+MSG (Group 6) showing seminiferous
236 tubule (T) with only few spermatogonia and lacking spermatids. H & E stains, ×400

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238 It is worthy of note that the marked increase in TAP and PAP activities noted in ARG+MSG
239 fed rats (Group 4) apparently supported the severe lesions observed in the testis sections of
240 the rats. This may underscore definitive adverse influence on the functional capacity of the
241 prostate and testis possibly due to negative interactive response following concomitant
242 ingestion of ARG and MSG in animals.

243 Although the present work was not designed to study possible mechanism(s), reports
244 especially that of Ross et al. [44], suggested that the endogenous level of androgenic
245 hormones (testosterone or dihydrotestosterone) may play a pivotal role in prostate disorders.
246 Hence, these agents may have elicited their effect via variable interference with one or more
247 of these androgenic hormones.

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249 **4. CONCLUSION**

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251 Collectively, these results seemingly indicate variable treatment related adverse effect on the
252 prostate function and the testis histology of the rats. The possible effect however, appeared
253 higher following concomitant exposure to ARG and MSG. Thus, caution should be exercised
254 in the simultaneous ingestion of arginine and monosodium glutamate in animals. **Further
255 research however, is required to [address some shortcomings (including small sample size)
256 of this study, and possibly],** elucidate the underlying molecular mechanisms of the present
257 observations in animal models.

258 **ACKNOWLEDGEMENTS**

259

260 The corresponding author gratefully acknowledges Dr. S. V. O. Shoyinka of the Department
261 of Veterinary Pathology, University of Nigeria, Nsukka for his kind assistance during the
262 histopathology studies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Obidoa O, Ezeanyika LUS, and Egbonu AC Cemaluk designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Egbonu AC Cemaluk and Ejikeme P Madus managed the analyses of the study, and managed the literature searches. All authors read and approved the final manuscript.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

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409

410 **DEFINITIONS, ACRONYMS, ABBREVIATIONS**

411 **DHT:** dihydrotestosterone

412 **PSA:** prostate specific antigen

413 **ARG:** L-arginine

- 414 **GLU:** L-glutamate
- 415 **MSG:** monosodium glutamate
- 416 **TAP:** total acid phosphatase
- 417 **PAP:** prostatic acid phosphatase
- 418 **PSA:** prostatic specific antigen
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