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Combined oral arginine and monosodium glutamate exposure induces adverse response on the prostate function and testis histology of rats

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ABSTRACT

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 to validate reliability.

Keywords: Testis histomorphology. Arginine. Glutamate. Prostatic acid phosphatase. Prostate.

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1. INTRODUCTION

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

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

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

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

2. METHODOLOGY

2.1. Animals and treatment

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

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

2.2. Sample collection and preparation

At the end of experiment, 15 h after the last feeding, the rats were sacrificed to obtain blood samples at 8:00 a.m, by retro orbital sinus venipuncture using sterile capillary tubes (containing no anticoagulant) as described by Egbuonu et al. [34], followed immediately by excision to obtain the testis samples. This study was carried out in accordance with ethical guidelines for animal welfare and approved by Biochemistry Department, Faculty of Biological Sciences, University of Nigeria Nsukka, Nigeria.

Blood samples were centrifuged for 10 min at 3,000g, room temperature, and the serum was stored in deep freezer for assays of biochemical parameters. Following excision, testis samples were collected immediately and fixed in 10% formaldehyde buffered saline (formal saline) for histological examination. The testis sections were stained and mounted using haematoxylin and eosin (H&E), as described earlier [35]. In brief, the testis specimens were dehydrated in graded levels of alcohol (70-100%) in ascending order to remove the water content. After dehydration, the tissues were cleared in xylene impregnated with paraffin wax and sectioned at 5 microns thickness using rotary microtone. The sections were floated on a water bath maintained at a temperature of 2-3^{o}C below the melting point of the paraffin wax after which the sections were dried on a hot plate maintained at a temperature of 2-3^{o}C above the melting point of the paraffin wax. After drying, the sections were stained and mounted using haematoxylin and eosin.

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

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

2.4. Statistical analysis

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

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Serum prostatic acid phosphatase (PAP) activity

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

3.1.2. Serum total acid phosphatase (TAP) activity

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

3.1.3. Histomorphological changes in the testis sections

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

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

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

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

3.2. Discussion

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

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

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

Groups	PAP activity (I.U/L) Mean ± S.D	TAP activity (I.U/L) 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*

Sample number, n = 4; * The mean difference is significant at the 0.01 and 0.05 levels.

a The mean difference is significant with other groups but not significant with control at 0.01 and 0.05 levels.

TAP and PAP activities of the various groups correlated positively at r (ρ)=0.01

This may be so since the elevation of serum level of these bio-markers was associated with prostatic cancer [26,27,28,44]. Thus, prostate dysfunction possibly induced by exposure to ARG, GLU or ARG+MSG may have resulted in the increased TAP and PAP levels noted in

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

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

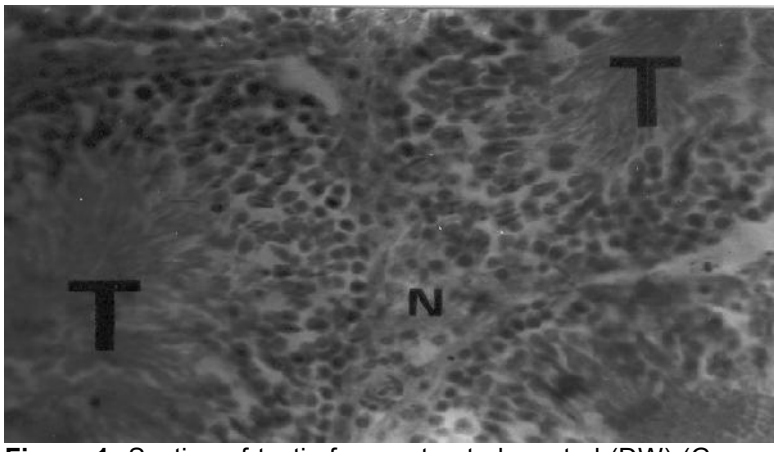

Figure 1: Section of testis from untreated, control (DW) (Group 1) rat showing semniferous tubules (T) and interlobular (interstices) spaces (N) showing normal Leydig cells. H & E stains, ×400

In particular, sections collected from rats treated with ARG showed histologic features similar to those of control (DW) rats, indicating non adverse influence on the testis following ARG exposure to rats. Earlier, Fahim et al. [46] reported no significant change in weight and histological structure of testes, epididymides, and seminal vesicles following exposure to ARG, even in combination with zinc.

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

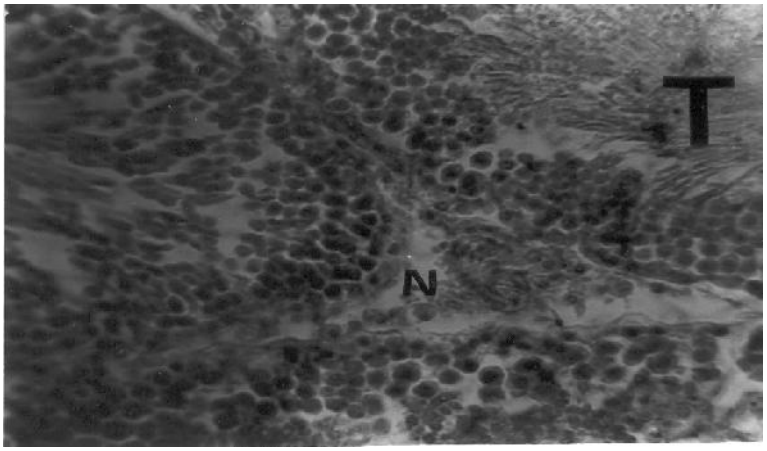

Figure 2: Sections of testis from rat treated with GLU (Group 3) showing semniferous tubule (T) with lots of spermatids, and oedematous interstitial space (N). H & E stains, ×400

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

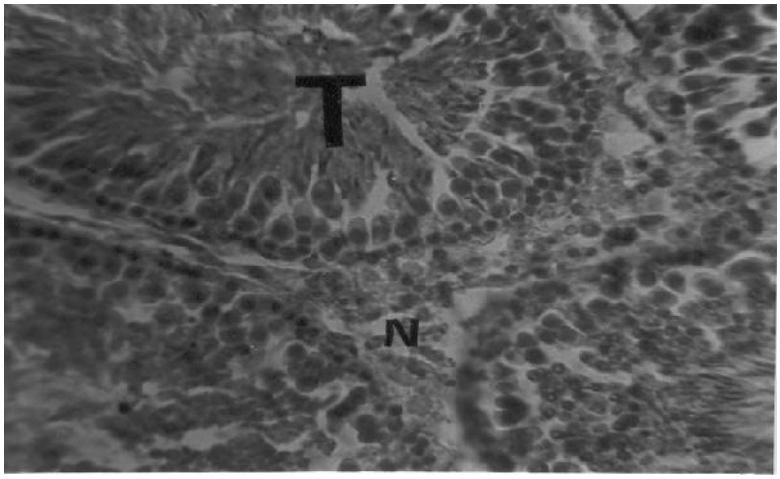

Figure 3: Section of testis from rat treated with MSG (Group 4) showing semniferous tubule (T) with only few spermatids and interstitial space (N) with inflammatory exudates. H&E stains, ×400

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

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

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

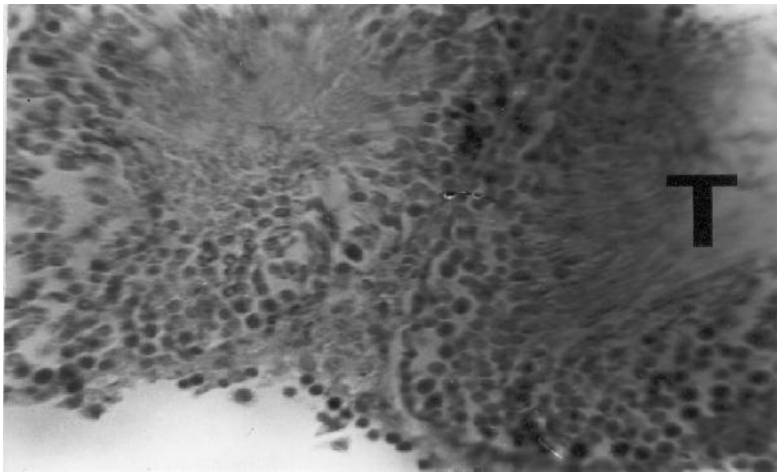

Figure 4: Section of testis from rat treated with ARG+MSG (Group 6) showing semniferous tubule (T) with only few spermatogonia and lacking spermatids. H & E stains, ×400

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

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

4. CONCLUSION

Collectively, these results seemingly 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 research however, is required (to address some shortcomings - including small sample size, of this study - to validate reliability, and to elucidate the underlying molecular mechanisms of the present observations in animal models).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Obidoa O, Ezeanyika LUS, and Egbuonu AC Cemaluk designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Egbuonu 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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DEFINITIONS, ACRONYMS, ABBREVIATIONS

DHT: dihydrotestosterone

PSA: prostate specific antigen

ARG: L-arginine

GLU: L-glutamate

MSG: monosodium glutamate

TAP: total acid phosphatase

PAP: prostatic acid phosphatase

PSA: prostatic specific antigen