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Green Tea Effects On the Semen Quality and Oxidative Stress of Adult Male Sprague Dawley Rats

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Abstract*— Green tea which has its origin in China is an aromatic beverage derived the withering and oxidation of Camellia sinensis leaves. It has been reported to have a wide therapeutic purpose; thus, greatly consumed. This study investigated the effects of the administration of various concentration of green tea on the semen quality and oxidative stress enzymes of adult male Sprague Dawley rats. The study was designed in line with the protocol and guideline of the Institutional Animal Care and Use committee (IACUC). Twenty (20) 58-62 days old male albino Sprague Dawley rats (Rattus Norvegicus), divided into five (5) animals per group were used for the experiment. Group 4; the control group received only distilled water, while Group 1, 2 and 3 were given 2ml/kg, 4ml/kg and 6ml/kg of aqueous extract of green tea respectively for 26 days. The animals were euthanized, the caudal epididymis was excised for semen analysis while the testis was homogenized for oxidative stress enzyme (Glutathione peroxidase and Malondialdehyde) assay. GraphPad Prism (version 8.0.2) Sidak's multiple comparison test was used to evaluate the differences in group means. The result from semen analysis.

Consumption of green tea at 2ml/kg does not disrupt the histoarchitecture of the testicular tissue; there was mild degeneration at 4ml/kg and 6ml/kg. There was simultaneous increase in the level of Glutathione peroxidase with the increase in concentration of green tea. There was a progressive increase for Malondialdehyde corresponding to a progressive decrease in the concentration of green tea. Thus, green tea helps to improve the quality of semen, reduction of fat and protection of the testicular tissue against oxidative stress.

Keywords*— Green tea, Histoarchitecture, Semen quality, Oxidative stress, Spraugue dawley rat.

I. INTRODUCTION

Plants are globally used as therapeutic agents since ancient time (D'cruzet al., 2010). Several plants are reported to enhance the reproductive process and some are known to hamper such functions. Green tea extract (GTE) has been used in traditional Chinese medicine for centuries to treat and prevent chronic diseases (Liao, 2007). Green tea is made from the steamed leaves and shrubs of camellia sinensis that have undergone minimal oxidation during processing (Liao, 2007). The processing originated in China but became associated with various cultures across the world. Green tea has recently become an extract which is used in health foods, beverages, dietary supplements and cosmetic items. Varieties of green tea have been created in the countries where it is grown. These varieties are due to the variability of the growing conditions e.g. horticulture, production, processing and harvesting time (Liao, 2007). Green tea, the minimally fermented (oxidized) product of the tea leaf, may show certain health benefits. In addition to their traditional use for making tea, the leaves are also individually processed. Many natural substances have been identified in green tea; green tea components theanine and catechins have neuroprotective functions (Yokogoshi et al.,1998; Kakuda, 2007). It has a significant role in the prevention of cancer, the catechins have been shown to inhibit tumor cell proliferation and promote destruction of leukaemia cells (Smith et al., 2001) and breast cancer cells (Masuda et al., 2002; Vergoteet al., 2002). Green tea was shown to decrease the risk of ovarian cancer (Zhang et al., 2002), in vitro studies show the proliferation of cervical (Ahn et al., 2003), prostate (Ahdani et al., 2003) cancer, head, neck (Masuda et al., 2002) and pancreatic carcinoma cells (Takada et al., 2002). It has been suggested that excessive intake of tea should be avoided by people who are prone to Anaemia (Samman et al., 2001). Green tea was shown to be an aromatase inhibitor in rat; a causative factor for an increase in testosterone level (Kao et al., 2000; Satoh et al., 2002). It has been reported that there was a decrease in plasma testosterone level by green tea epigallocatechin- gallate (EGCG). Goitrogenic/antithyroidal effect of GTE, in relatively high doses has been reported in both in vivo and in

vitro studies (Chantra et al., 2010) and the role of thyroid hormone on the growth and normal functioning of the male gonads are well documented (Longscope, 2008). Green tea is marketed commercially in Nigeria as tea bags, in various brand names. Green tea can taste sweet, bitter or astringent depending on the temperature of the water 60oc water for sweet 80oc for astringent and 100oc of water for bitter tea respectively.

II. MATERIALS AND METHODS

2.1 Experimental design

Twenty 58-62 days old male albino Sprague Dawley rats (Rattus Norvegicus), with weights ranging from 90g to 200 g were used in this study. Animals were maintained in line with national guidelines and protocol of the Institutional Animal Care and Use committee (IACUC). Animals were housed in clean net cages with adequate space to enhance free movement. The environmental condition was kept relatively constant with good lightening and temperature in the animal house of the Anatomy Department of the University of Ilorin and this was maintained throughout the course of the experiment and water given ad lib.

The green tea was acquired from a tea store in Ilorin, available in tea which is the commercial version that was prepared according to the method devised by Ganza and Regula (Yuan et al., 2010). Each bag of green tea has a net weight of 2g each, the aqueous extract was prepared in three different concentrations with 100ml of boiling distilled water as the solvent; low concentration (2 g of green tea), moderate concentration (4g of green tea), high concentration (6g of green tea). The aqueous extract was obtained by boiling the distilled water up to 100oC, the boiling water is then poured into a cup which contained the green tea bag and allowed to stand for 4mins. This procedure is repeated for each of the concentrations differently.

The animals were divided into 4 equal groups of 5 rats per group according to their average body weight. Groups 1(A)-3(C) were the treated groups and group 4 was the control group. The administration which was carried in the early hours of the morning, before feeding lasted for 26days. Group A received 2ml/kg the low concentration of aqueous green tea extract (AGTE), group B was given 4ml/kg of the concentration AGTE, group C was given 6ml/kg of the high concentration of the AGTE, and the control was given 2ml/kg of distilled water throughout the experimental period. The extract was administered orally using the oropharyngeal cannula and a syringe (2.0ml). In accordance with the IACUC regulation, animals were euthanized on the 27th day following administration of the AGTE after which they were dissected and the testes were excised for tissue processing while some were homogenized for enzyme assay. Semen was collected from the caudal epididymis for immediate semen analysis.

2.2 Estimation of sperm motility

Principally, the motility of spermatozoa depends on the development of the axoneme structures (for example; microtule), the presence of the mitochondria sheathes and the implantation of the flagellum at the nucleus by both centrioles. This allows the full estimation of expression of the rotating movement of normal spermatozoa. Both motile and non-motile are calculated from the mean value. The value is then adjusted to the closest five. Mean quality of sperm is graded as A, B, C, D depending of the following;

a. Rapid progressive motility --------- A.
b. Slow progressive motility ---------- B.
c. Non progressive motility ---------- C.
d. Immobility ------------------------- D.

2.3 Enzyme assay

Glutathione Peroxidase (GPx) was determined using (Paglia et al. (1967) method. The homogenate (sample) was allowed to attain room temperature. An aliquot quantity of the homogenate was centrifuge at 5000g for 5min. 25 microlitre of the centrifuged sample was pipetted into 0.5ml of glutathione peroxidase diluting agent.

Sample	**Blank**	
Diluted sample (homogenate)	20µl	20µl
Distilled water	-	-
Reagent R1 (Reduced Glutathione in buffer)	1.0ml	1.0ml
Reagent R2 (cumenehydroperoxide)	40µl	40µl

The timer was started simultaneously with the addition of R2; the spectrophotometer was blanked with distilled water. The initial absorbance of both the test and blank was read after 1min and again after

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1 and 2 minutes at 340nm. The rate of charge is absorbance for both blank and test was calculated. The blank value was subtracted from that of the sample (test) to arrive at the actual rate of change in absorbance for the test (Δabs).

Unit of enzyme activity/L of diluted homogenate = (8412 xΔ abs)

Enzyme activity in Unit/L of homogenate = (8412 x ΔAbs) × 21 [Note: 21 was the dilution factor].

Malondialdehyde (MDA) in the specimens were measured according to the protocol outline by Stocks and Domandy (1971) as shown below.

- 0.1ml of homogenate was pipetted into a plastic test tube
- 1ml of 20% Trichloroacetic was added to it. The mixture was mixed and centrifuged at 2000g for 5mins.
- 0.5ml of the supernatant was pipetted into a Pyrex test tube.
- 0.05ml of 5.0µmol/L of 1,1,3,3 – Tetramethoxyl propane was pipette in to another pyrex test tube (STD).
- 0.5ml of Trichloroacetic acid solution and 1.0ml of Thiobarbituric acid was pipette into a 3rd pyrex test tube (Blank)

All tubes were stoppered tightly, heated in a water bath at 100oC for 20minutes, and then allowed to cool in water. The spectrophotometer was blanked using the reagent blank at 532nm. Absorbance of tests and standard were read (Stock and Domandy, 1971).

III. RESULTS

The results from the analysis were presented as mean±S.D. Table 1 represents the mean values for the semen quality analysis, while Fig. 2 present the comparison of the mean values for the glutathione peroxidase (GPx) and malondialdehyde (MDA) levels in the control and experimental groups.

3.1 Semen quality

The

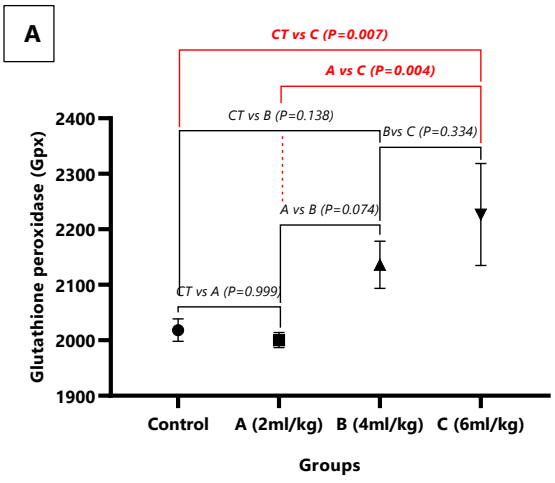
Table 1: The parameters for semen quality analysis and test of difference in the groups.

Parameters	Control	Group A	Group B	Group C
Sperm Count (×10^6)	70.70±4.30	65.40±8.40*†	64.40±6.00*†	63.40±1.40*†
Motility (%)	86.55±2.55	85.35±0.05*	89.10±2.10*†	87.30±1.20*
Morphology (%) (Normal)	83.25±2.85	82.60±0.50*†	82.85±1.55*†	83.45±1.35 †
Life/Death ratio (%)	89.15±0.55	90.40±0.80	91.55±0.45*†	89.35±0.50
Progressivity	*B*	*A/B*	*A*	*A*

Note: *Statistically not significant with the control, † Statistically not significant between the treated groups

3.2 GPx and MDA levels

The mean GPx level in the control group was lowest, with a steady increase for group A (2ml/kg); with Group C (6ml/kg) having the highest mean GPx level while Group B (4ml/kg) had the second highest mean value. However, the observed differences between the groups were not significant (p>0.05) (Fig. 2). The level of malondialdehyde was highest in the treated group 1, the level of MDA in control group was however higher when compared to the treated group A, C, and B with the lowest value observed for group A, although statistically there was no significant difference at (P>0.05) (Fig. 2-A & B).



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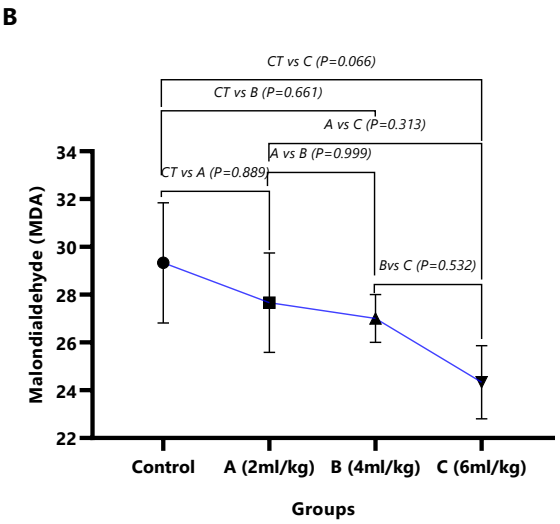

Figure 2: Comparison of the (A) Glutathione peroxidase (Gpx) and (B) Malondialdehyde (MDA) levels in the groups

3.3 Morphological observations of the spermatozoa
The administration of 2ml/kg, 4ml/kg and 6 ml/kg of aqueous extract of green tea (GT) to the treated groups of the experimental animals compared to the control group has effect on the structure of the sperm produced by the testis and hence the marked morphological changes. There was no evidence of gross morphological changes observed during the removal and physical examination of the testis, and when the caudal epididymis was excised for semen analysis there was also no any marked gross morphological changes. Microscopically, the morphology of the group treated with 4ml/kg and 6ml/kg of GT were better when compared to the control and group treated with 2ml/kg of green tea; however, the number of abnormal sperm (double head) in group B & C increased.

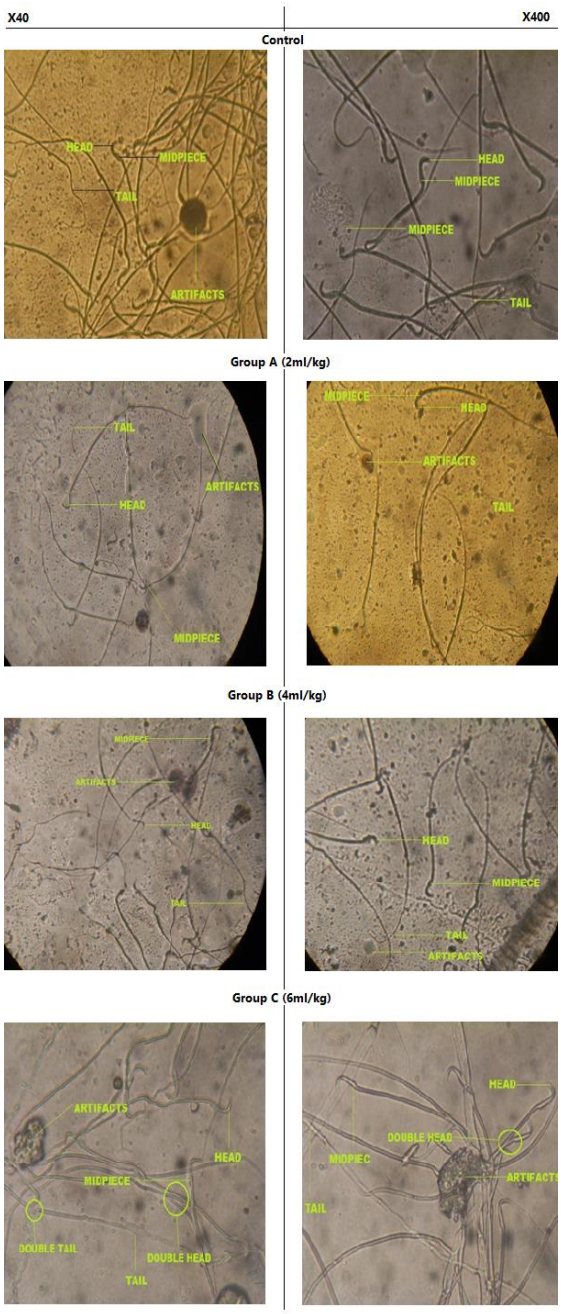

Figure 2: Photomicrograph of the transverse section through the caudal epididymis the control and experimental groups (MG X40 AND 400).

DISCUSSION

Previous researches showed that the harmful effects of green tea consumption are not as much compared to the beneficial and the therapeutic effects. Administration of green tea extract orally, at relatively low, moderate and high concentrations at a dose of 2ml/100g body weight daily for 26days, reduced the net weight gain in body weight of treated animals in comparison to that of their respective control animals. This is in agreement with

what was reported earlier by (Kao et al., 2000; Sayan et al., 2000) after the administration of green tea extract (GTE), there was a net decrease in weight gain due to the actions of polyphenols. The proposed action of green tea on the decrease of body weight is due to the inhibition of catechol-o-methyl transferase (COMT) enzymes by EGCG of the tea (Dulloet al., 1999; Dulloet al., 2000; Chantreet al., 2000). It has been shown that thermogenesis and fat oxidation are stimulated by norepinephrine, the action of which is degraded by the enzyme COMT (Dulloet al., 2002). Therefore, the inhibition of COMT by EGCG decreases body weight. However, no such changes well recorded after the dose of the low concentration of GTE was used in this study.

The parameters evaluated include sperm count, motility, morphology, life-death ration and progressivity. Although sperm motility and sperm count were highest for control and group 1, when subjected to statistical analysis at ($p>0.05$) the difference between the control and the treated group was not significant. Sperm morphology observations showed different abnormal sperms, for example, sperms with abnormal shape of head (curved, round and double) and tail (short, double and long) were observed, especially in group 2 and 3 which received the moderate and high concentration of GTE but statistical analysis at ($p> 0.05$) shows that the differences in morphology was not significant comparing within the experimental group and with the control group. The control and group 1 have more life sperm than the group 2 and 3 but statistical analysis at ($p>0.05$) shows that the difference in the life-death ratio was not significant. The progressivity has to do with the forward directional movement of the sperm and different grading Parameters were used. The control and group 1 had rapid progressive motility while group 2 and 3 has a slow progressive motility. This is in agreement with was reported earlier by Chandra et al. (2010) that consumption of green tea at a moderate rate improves the semen quality (count, motility, morphology etc).

Group 1 showed similar characteristic as the control group when the photomicrograph of the cross section of the seminiferous tubule was observed. The photomicrograph of groups 2 and 3 showed mildly degenerated testicular tissue. This shows that green tea consumption at a moderate rate improves the reproductive characteristics of the male gonads, consistent with the work done by Chandra et al. (2010), which elucidates the effects of green tea on the morphology of the testis.

The DNA Feulgen reaction is well demonstrated in the spermatogenic cells by the dark-reddish – coloured stain which indicates Feulgen positivity. The intensity of the stain is more in the control group and group 1. The intensity of the stain is however lower in group 2 and 3 compared to the control group and group 1 which received the lower concentration of green tea. The intensity of the stain represents positivity of the Feulgen reaction; hence the presence of more DNA. This enhances spermatogenesis and the basis for the good outcome of the semen analysis in group 1 and control group also in agreement with the work done by Chandra et al. (2010).

GPx is an antioxidant that belongs to the family of Selenoproteins. It plays an important role in the defense against oxidative stress. It is vital in the eradication of free radicals of oxygen in the body by releasing glutathione (Cabrera, 2006). The increase in the level of glutathione is as a result of the corresponding increase in the green tea concentration, this shows the antioxidant power of green tea, corroborating the work done by Cabrera (2006) when he elucidated the antioxidant effects of green tea.

Malondialdehyde is one of the most frequently used indicators for lipid peroxidation, it causes toxic stress in cells and the production of this aldehyde is used as a biomarker to measure oxidative stress in an organism (Moore et al., 1998; Del et al., 2005). It is a mutagenic due to its reactivity (Hartman, 1983). The high level of MDA recorded in group 1 given 2ml/kg (low conc.) of green tea and the control group given distilled water respectively for 26days signifies that the oxidative stress in these groups was high unlike the group 2 and 3 which received 4ml/kg and 6ml/kg of green tea respectively, these groups had abundance of antioxidants from the green tea hence the low level of MDA. Antioxidants are responsible for mopping up free radicals of oxidation. This corroborates what was reported by Del et al. (2005) and Cabrera (2006) when they elucidated the relationship between the toxic effect of Malondialdehyde and its role as a biomarker for oxidative stress and the counteractive effects of green tea in mopping up the free radicals released.

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