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Effects of Smoothies on Oxidative Stress Markers Following Administration of Monosodium Glutamate in Male Wistar Rats

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Abstract

Fruits are medicinal and also contain essential phytonutrients that gives the fruits potency that keeps the body healthy. The aim of the study is to ascertain the Effects of Smoothies (banana, apple and pineapple) on Oxidative Stress Markers following administration of monosodium glutamate in Male Wistar Rats. Thirty (30) animals weighing 130kg to 180kg were randomly selected into 6 groups with 5 animals per group. Group 1 received 5mls of distilled water, group 2 received 1ml/kg (low dose) of smoothies, group 3 received 2ml/kg (medium dose), group 4 received 3ml/kg (high dose), group 5 received 400mg/kg of monosodium glutamate, group 6 received 400mg/kg of monosodium glutamate and 3ml/kg (high dose) of smoothies co-administered. Administration was carried out for 14 days and on the 15th day, the animals were sacrificed, semen was harvested and 5ml blood was collected via cardiac puncture. Statistical analysis was done using ANOVA and expressed as Mean±SEM. Statistically $P < 0.05$ was said to be significant. SPSS version 26 was used. The results showed significant increase in SOD in the group that was administered smoothies (high dose) and decrease in the group that was administered 400mg of monosodium glutamate (MSG). MDA increases in the groups that was administered MSG alone and when co-administered with smoothies (high dose). MDA also decreases in the groups given medium and high dose smoothies. Catalase decrease in the group that was given MSG alone. Gluthione increases in the groups that was given medium and high dose smoothies and decrease in the group that was given MSG only.

Keywords: Effects, smoothies, oxidative stress markers, administration, monosodium glutamate.

INTRODUCTION

The production of free radicals is a double-edged sword in reproduction system (Silva *et al.*, 2010). Physiological concentrations of free radicals are required to mediate normal processes of capacitation, hyperactivation, acrosome reaction, fertilization, and embryo development (Rhee 2006; Desai *et al.*, 2009; Gonçalves *et al.*, 2010). However, above-physiological levels of free radicals can result in oxidative stress which lead to sperm or ovum damage, deformity, endometriosis, pre-eclampsia, miscarriage, intrauterine growth retardation, and infertility (Agarwal *et al.*, 2005; Bansal and Bilaspuri 2010). Under normal physiological conditions, living organisms possess a wide range of enzymatic antioxidants, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) (Szczepanska *et al.*, 2003; Celino *et al.*, 2011), and non-enzymatic antioxidants, including glutathione (Gul *et al.*, 2000), uric acid, and coenzyme Q to scavenge excess free radicals (Agarwal *et al.*, 2003). But under pathophysiological conditions, endogenous antioxidants may not counteract excess free radicals. Hence, there is

a continuous demand for exogenous antioxidant supplementation (Lykkesfeldt and Svendsed 2007).

Many natural plants and their seed, leaf, or root extracts which are rich in polyphenols, flavonoids, carotens, gallic acid, tannins, and essential oil as antioxidants have been recognized to be better than synthetic antioxidants due to lower cytotoxicity and residue (Gupta and Sharma 2006; Nagulendran *et al.*, 2007; Sen *et al.*, 2010).

Physiological levels of ROS play important roles in male animal reproduction system (Agarwal *et al.*, 2008). Moderately elevated concentrations of ROS induce sperm immobilization via depletion of intracellular ATP and subsequent decrease in the phosphorylation of axonemal, but over physiological levels of ROS induce lipid peroxidation and result in sperm cell death (Misro *et al.*, 2004). Spermatozoa membranes are vulnerable to free radicalinduced damage because they are not only rich in polyunsaturated fatty acids (Maneesh and Jayalekshmi 2006), but also contains low concentration of antioxidant enzymes (Sawyer *et al.*, 2001; Saleh and Agarwal 2002; Maneesh and Jayalekshmi 2006).

Furthermore, spermatogenesis in testes is an extremely active replicative process to generate sperm at a high rate. This high rate of cell division is accompanied with high production of free radicals due to high amounts of mitochondrial oxygen consumption by germinal epithelium (Aitken and Roman 2008). Hence, an imbalance of free radical generation and detoxification cause oxidative stress and damage to cellular lipids, proteins, amino acids, sugars, nucleic acids, and midpieces in sperm and testicular tissues which lead to subsequent poor semen qualities. Poor semen qualities account for more than 80% failure of fertilization and embryogenesis, miscarriage, and infertility in male animals (Gadea and Matas 2000; Rabbani *et al.*, 2010; Enciso *et al.*, 2011).

Spermatogenesis depends on intratesticular and extratesticular hormonal regulatory processes and functions of the intertubular microvasculature (Holstein *et al.*, 2003). Semen parameters such as sperm count and concentration, viability, mobility, and morphology are indicators to evaluate semen functions (Huynh *et al.*, 2000; Rodriguez-Martinez 2003, 2006).

Oxidative stress is a main underlying cause which can interfere with spermatogenesis, reduce sperm quality and production, and even cause infertility (Boonsorn *et al.*, 2010). Because elevated ROS generation causes damage to the spermatozona DNA, results in increased apoptosis of cells, and therefore, leads to a low fertility rate (Kaur and Bansal 2003). The application of exogenous plant derived antioxidant is likely to improve health status of male animals (Agarwal and Prabakaran 2005; Nantia *et al.*, 2009). Numbers of flavonoid-containing plants are known to have antioxidant, androgenic, and anti-infertility activities and have been extensively used against animal reproductive diseases (Middleton *et al.*, 2000; Dobrzyñska *et al.*, 2004; Purdy *et al.*, 2004).

Phytomedicine involves the use of various plant's parts such as leaves, stems, seeds, fruits, barks and roots to treat certain disease at home. Different plants show different phytochemical constituent while some shows similar phytochemical constituents (Gbaranor *et al.*, 2021). In addition to natural herbaceous plants, some fruit and vegetable extracts with antioxidant properties display beneficial effects in animal reproduction system. Nevertheless, some plants, despite containing antioxidant substances, display detrimental effects and therefore cause defects and reproductive failure in male animals (Knight and Walter 2004). The dose-dependent manner may explain such dual functions (Na and Surh 2008; Moskaug *et al.*, 2005).

Monosodium Glutamate (MSG) is widely used across the globe as food flavour's enhancer. Monosodium glutamate (MSG) is known to induced oxidative stress by causing lipid peroxidation, decrease the level of glutathione and also caused the rise of the activities of glutathiones- transferase, catalase and superoxide dismutase (Onyema *et al.*, 2006).

MATERIALS AND METHOD

Fruit Collection and Identification

Fresh fruits were obtained from mile 3 market in the month of October, 2023. Identification of the fruits was done by Dr. M. G. Ajuru of the department of plant science and biotechnology, Faculty of Science, Rivers state university. The fruits were registered with the code number RSUHPb0155 for banana, RSUHPb0154 for pineapple, and RSUHPb0153 for apple.

Fruit Preparation

Washed banana, pineapple and apple fruits were cut into medium sizes and Poured the fruits into the fruit blender and plugged the blender to an electric source. 250ml of water was added and blended for 5 minutes until it becomes smooth. The blender was Unplugged from electric source and the smoothly blended smoothies was turned into a beaker and used for administration.

Animal and Management

A total of thirty (30) male Wistar rats between 130g to 180g of weight were obtained from animal facility, Faculty of Basic Medical Sciences, Rivers State University, Port Harcourt. The rats were housed under standard laboratory conditions with controlled temperature, humidity. They were provided with standard rat chow and water. The animals were weighed before and after administration of smoothies.

Study Design

The rats were randomly selected into six (6) experimental groups with five (5) rats per group and administration of substances for 14 days.

Group 1: was administered 5ml of distilled water for 14 days

Group 2: was administered 1ml of smoothies (low dose) for 14 days

Group 3: was administered 2ml of smoothies (medium dose) for 14 days

Group 4: was administered 3ml of smoothies (high dose) for 14 days

Group 5: was administered 400mg of monosodium glutamate for 14 days

Group 6: was administered 400mg of monosodium glutamate + 3ml of smoothies (high dose) for 14 days

Sample Collection

Administration of smoothies (banana, apple and pineapple) was done for 14 days and on the 15th day, the animals were anaesthetized with chloroform soaked in cotton wool and thereafter, sacrificed.

Blood Collection

5mls of blood was collected via cardiac puncture using syringe and transferred into an EDTA bottle and used to determine Oxidative stress markers.

Statistical Analysis

Statistical analysis was done using ANOVA and expressed as Mean±SEM. The statistically p less than 0.05 was said to be significant. SPSS version 26 was used.

Ethical Approval

Application for ethical approval to the Research Ethics Committee, Faculty of Basic Medical Sciencies, Rivers State University was approved with the number RSU/FBMS/REC/23/026.

Superoxide Dismutase (mista and fridouich method)

Principle: The activity of SOD to inhibit the auto-xidation of epinephrine at PH 10.2 make this reaction a basis for a simple assay for this dismutase.

Procedure: 0.2ml of sample was diluted in d/w to make a 1:10 dilution, 200ul of the diluted sample was added to 2.5ml of 0.05m carbonate buffer (pH10.2). Then started the reaction by adding 0.3ml of freshly prepared 0.3mm epinephrine to the mixture, which was quickly mixed by inversion. Read and recorded increase in absorbance at 480nm for 30secs to 2.5mins. Unit: u/ml

Catalase Estimation (Clairborne method)

Principle: catalase in the sample split hydrogen peroxide which is measured at 240nm. One unit of catalase activity equal the amount of protein that convert 1umol H_2O_2/min.

Procedure: 0.2ml of sample was added to phosphate buffer containing 100mm of H_2O_2, in a total of 1ml. Incubated for 2mins at 37'c. Read and recorded change in absorbance at 240nm, unit u/g

Malondialdehyde (MDA) Ohkawa & Ohishi method

Principle: Under acidic condition, MDA produced from the peroxidation of facty acid membranes react with the chromogenic rgt, 2- thiobabituric acid to yield a pink coloured complex which is measured at 532nm. unit umol/ml

Procedure: An aliquot of 0.4ml of supernatant was in mix with 1.6ml of Tris-Kcl buffer to which 0.5ml of 30% TCA was added. Then 0.5ml of 0.75% TBA was added and placed in a boiling water for 1hr. This was the cooled in ice and centrifuged at 4000 rpm. The clear supernatant was collected and the absorbance measured at 532nm using d/w as blank.

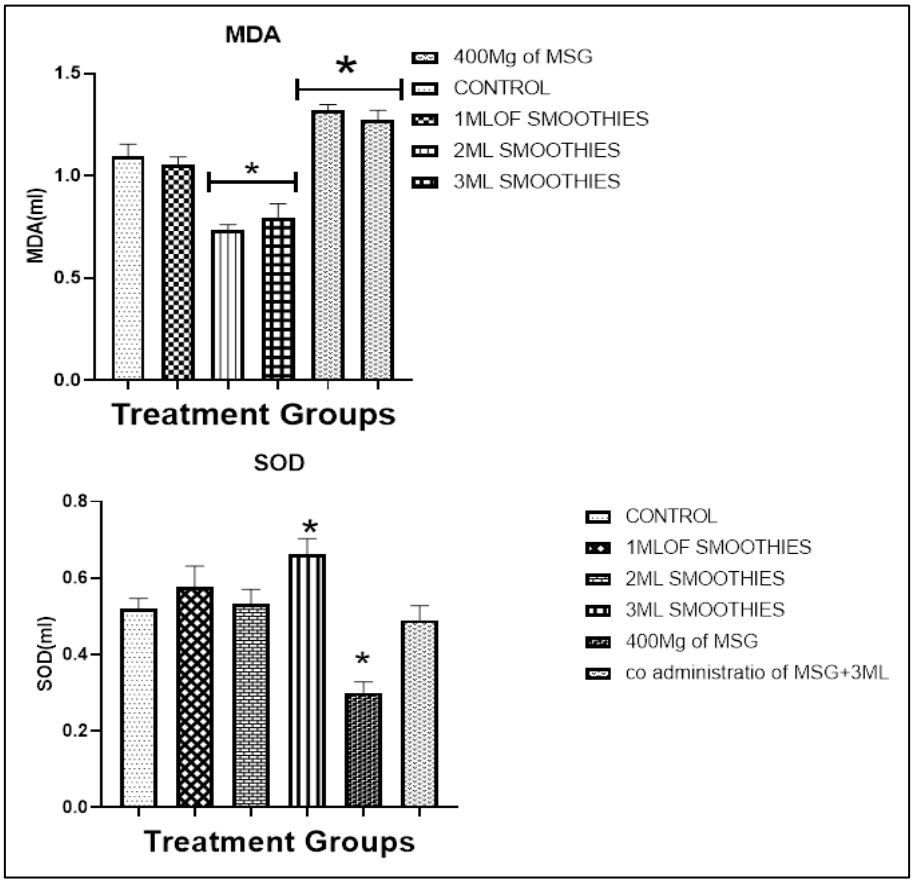
Glutathione (sedlak & Lindsay method)

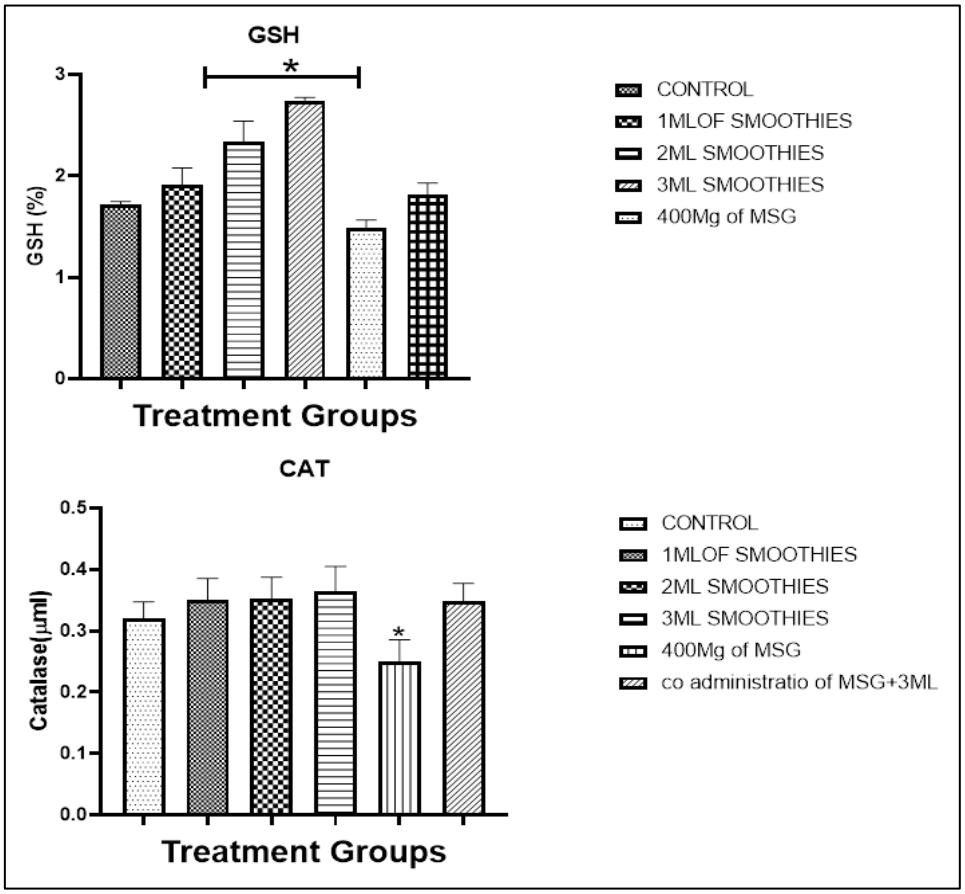
Principle: The reduced form of glutathione comprised in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable yellow colour when 5,5 dithiobis -2- nitrobenzoic acid (Ellman's rgt) is added to sulfhydryl- compounds. The chromophoric product resulting from the reaction of Ellman's rgt with the reduced GSH is measured at 412nm.

Procedure: 0.2ml of the sample was added to 1.8ml of d/w and 3ml of the precipitating solution and mixed. Allow to stand for 5 min and centrifuged at 4000rpm for 10mins. 1ml of filtrate was added to 4ml of 0.1m phosphate butter. 0.5ml of DTNB was finally added. Read and record the absorbance at 412nm using a prepared blank to 3ero the spectrophorometer. unit ug/ml

RESULTS

The results showed significant increase in SOD in the group that was administered smoothies (high dose) and decrease in the group that was administered 400mg of monosodium glutamate (MSG). MDA increases in the groups that was administered MSG alone and when co-administered with smoothies (high dose). MDA also decreases in the groups given medium and high dose smoothies. Catalase decrease in the group that was given MSG alone. Gluthione increases in the groups that was given medium and high dose smoothies and decrease in the group that was given MSG only.


MDA
400Mg of MSG
CONTROL
1MLOF SMOOTHIES
2ML SMOOTHIES
3ML SMOOTHIES
MDA(ml)
1.5
1.0
0.5
0.0
*
*
Treatment Groups
SOD
CONTROL
1MLOF SMOOTHIES
2ML SMOOTHIES
3ML SMOOTHIES
400Mg of MSG
co administratio of MSG+3ML
SOD(ml)
0.8
0.6
0.4
0.2
0.0
*
*
Treatment Groups


GSH
CONTROL
1MLOF SMOOTHIES
2ML SMOOTHIES
3ML SMOOTHIES
400Mg of MSG
GSH (%)
3
2
1
0
*
Treatment Groups
CAT
CONTROL
1MLOF SMOOTHIES
2ML SMOOTHIES
3ML SMOOTHIES
400Mg of MSG
co administratio of MSG+3ML
Catalase(µml)
0.5
0.4
0.3
0.2
0.1
0.0
*
Treatment Groups

DISCUSSION

Fruits are natural and are eaten every day as food or are use as medicinal to improve the health status of an individual. These fruits come in various size and shapes and its contain phytonutrients which carry out the role of the fruits.

The results of the findings showed that superoxide dismutase (SOD) levels in the group treated with 3mls/kg of smoothies increases significantly when compared with control. This increase in the levels of SOD at dose of 3mls/kg shows that the smoothies may protective. In the group given MSG 400mg/kg has its SOD decrease significantly and this is in consonant with several previous studies that MSG induces oxidative stress. Catalase levels was significantly high in the treated groups 1ml/kg, 2mls/kg 3mls/kg and 3mls/kg plus MSG 400mg/kg co-administered when compared with control. This increase in the catalase levels was not significant and this could be due to time dependent. In the group treated with MSG 400mg/kg, the catalase levels significantly decrease when compared with the control and thus shows that MSG is implicative. Malondialdehyde (MDA) significantly reduce in the groups treated with smoothies 1ml/kg and 3mls/kg when compared with the control. This decrease in the level of MDA shows the smoothies is protective against oxidative stress. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells and an increase in the free radicals causes overproduction of MDA. Malondialdehyde level is commonly refer to as marker of oxidative stress. However, the group treated with MSG 400mk/kg and the group co-administered with smoothies 3mls/kg and MSG 400mg/kg has its MDA levels significantly increase when compared with control group. Gluthione (GHx) level significantly increase in 2mls/kg and 3mls/kg treated groups when groups and it is protective. GHx level significantly decrease in the group administered with MSG 400mg/kg and this indicate that MSG induces oxidative stress. The study revealed that smoothies is protective against oxidative stress.

CONCLUSION

Fruits are important, natural and its contained phytonutrients that play a vital role. The findings revealed that smoothies are protective against oxidative stress due to increase levels of SOD, CAT and GHx and decrease level of MDA.

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Ethical approval: Required

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