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Antioxidant effects of ethanol seed extract of *Mucuna Sloanei* (Hamburger Bean) on Monosodium Glutamate intoxicated albino rats

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ABSTRACT

Some antioxidant effects of *Mucuna sloanei* ethanol seed extract (MSEE) were investigated in monosodium glutamate (MSG) intoxicated rats. Thirty (30) female albino rats were randomly divided into six groups (n=5) and treated once per day *per os* for 28 days as follows; Group A received only clean water; Group B was intoxicated with MSG at the dose of 8000 mg/kg b.w only; Group C received 400 mg/kg b.w of the extract only; Groups D-E were intoxicated with MSG (8000 mg/kg b.w) and also received different doses of the extract; 200, 400 and 800 mg/kg b.w respectively. Thereafter, blood was collected for assay of reduced glutathione GSH, superoxide dismutase (SOD) and catalase (CAT) activities, as well of malondialdehyde (MDA). The results showed that MSG reduced GSH level from 63.08±3.98 IU/L in rats given only MSEE to 37.37±2.27 IU/L. This was however reversed by MSEE in intoxicated rats at all doses, with 400 mg/kg giving a significant ($P<0.05$) increase in GSH of 50.25 IU/L. The extract at 800 mg/kg, significantly ($P<0.05$) increased SOD and CAT activities from 20.15 IU/L in MSG intoxicated rats to 25.43 IU/L, and 3.75 IU/L to 29.80 IU/L respectively. The extract also reduced the level of malondialdehyde, a marker for peroxidation, which was increased by MSG intoxication. We conclude that MSEE reverses oxidative effects of MSG in intoxicated rats.

Keywords Antioxidant, *Mucuna sloanei* seeds, monosodium glutamate, intoxication

INTRODUCTION

Antioxidants prevent oxidation in cells by mopping up the free radicals from endogenous and exogenous processes (Bouayed and Bohn, 2010). The antioxidants from diets include precursors like vitamins A, C, E and minerals such as selenium and zinc which are not necessarily antioxidants but are referred to as antioxidant nutrients needed for the proper functioning of the antioxidant enzymes (Davis *et al.,* 2012). The body produces enzymes which function as endogenous antioxidants, including superoxide dismutase, glutathione, catalase and hydrogen peroxidase. The exogenous antioxidants function synergististically with the endogenous antioxidants in reducing the body's oxidative stress (Bilici *et al.,* 2001). *Mucuna sloanei* is a plant known to have some medicinal properties. It is a climbing shrub seen mainly around swampy areas. The seeds are contained in a pod containing two or three seeds. These seeds have a characteristic three-layered appearance, appearing like the eyes of a large mammal or like hamburger leading to names like "Oxeye bean" or "Hamburger bean". The seeds can be polished and made into seed necklaces (Diallo *et al.,* 2002). *Mucuna* seeds are usually toasted before grinding and flouring to supplement as thickener in sauce or soups (Ezueh, 1997; Waryekeche *et al.,* 2003). *Mucuna sloanei* seed is used by the Igbo community in Sub-Saharan Africa as a condiment or part of the main dish as soup thickener (Ukachukwu *et al.,* 2002).

Traditionally, the seeds have been used as a diuretic, purgative and for soothing haemorrhoids (Standley, 1926). The seeds are also used to make black dye and to produce oil that can be used for various purposes such as soap. The constituents of *Mucuna sloanei* seeds include crude proteins, carbohydrates, fat, crude fibers, moisture, ash, phosphorus, magnesium, calcium, sodium, iron, manganese, copper, tannins, glycosides, zinc and L-Dopa (Tuleun *et al*., 2008; Nwosu, 2011). Its medicinal properties include antidiabetic and antiparkinsonism (Molloy *et al*., 2006), anti-oxidant and antimicrobial (Rajeshwar *et al*., 2005), aphrodisiac, antineoplastic, anti-epileptic, enhances learning and memory (Poornachandra *et al*., 2005) and antihelminthic (Jalalpure, 2007).

Monosodium glutamate is a food additive used to improve the flavour of foods with an umami taste (Ikeda, 2002). It is a flavor enhancer and serves to balance, blend, and round the perception of other tastes (Loliger, 2000). It has also been demonstrated that high concentrations of monosodium glutamate in the central nervous system induce neuronal necrosis and damage in retina and circumventricular organs. Studies have shown that MSG produces oxygen derived free radicals (Singh and Ahluwalia, 2003) and its chronic administration induced oxidative stress in experimental animals causing retinal degeneration, endocrine disorder, addiction, stroke, epilepsy, brain trauma, neuropathic pain, schizophrenia, anxiety, depression, Parkinson's disease and Alzheimer's disease. (Adrienne, 1999; Eweka and Adjene, 2007). The aim of this study, therefore, is to determine the antioxidant effects of the ethanolic seed extract of *Mucuna sloanei* on monosodium glutamate intoxicated albino rats.

MATERIALS AND METHODS

Collection and preparation of *M. sloanei* ethanol seed extract (MSEE)

Mucuna sloanei seeds were obtained from Amokwo Ugwu Nkpa in Bende LGA in Abia State and authenticated at the Department of Crop Science, College of Crop and Soil Science, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. A voucher specimen was deposited in the herbarium of Veterinary Physiology and Pharmacology laboratory, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The seeds were toasted, dehulled and ground. A portion (500 g) of the powder was extracted in 2.0 litres of 98 % ethanol for 48 hours agitated intermittently every 3-hours using cold maceration method, after which it was sieved using Whatman's® filter paper (No 4.) and dried using hot air oven set at 40^{o}C. Calculated amount of the extract was weighed and reconstituted in distilled water to give the required different doses of 200, 400 and 800 mg/kg body weight and given per os using oral gavage daily for 28 days. The acute toxicity study (LD_{50}) showed that *Mucuna sloanei* extract was not toxic with LD_{50} of 4000 mg/kg b.w. (Oguwike *et al*., 2017),

Monosodium glutamate intoxication

Monosodium glutamate (Ajinomoto®) was procured from Ubani market Umuahia, Abia State. A toxic concentration of 500 mg/ml was formulated to be given to all the groups at the dose of 8000 mg/kg except Group A (Plain water only) and Group C (Extract only) per os using oral gavage daily for 28 days.

Experimental Animals

Thirty female albino rats (*Rattus norvegicus*) of Wistar strain with an average weight of 113 g were obtained from the Animal house of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. They were housed in aluminum cages under standard conditions (temperature 26 °C; photoperiod-12 hours day light and 12 hours darkness; humidity: 45-50%) and allowed to acclimatize for two weeks before the experiment. They were given free access to feed (Grower pellets from Vital Feeds®, Nigeria) and clean tap water. All the animals received humane treatment in accordance with the guidelines of the National Institute of Health, USA for ethical treatment of laboratory animals.

Experimental design

Thirty (30) mature female albino rats were randomly divided into six groups (n=5) and dosed once per day for 28 days as follows; Group A (Control) received 1 ml of plain water as sham control; Group B received 8000 mg/kg b.w of MSG only; Group C received 400 mg/kg b.w of the extract only and Groups D-F received 8000 mg/kg b.w of MSG with different doses of the extract (200, 400 and 800mg/kg b.w) respectively. Dosing was done *per os*. On day 29, the rats were sacrificed by inhalation anaesthesia using chloroform. Blood samples were collected from the rats in all the groups from the heart through abdominal dissection under chloroform anaesthesia.

Estimation of reduced glutathione (GSH) activity

Reduced glutathione was determined by the method of Ellman (1959). A volume (0.1 ml) of serum was treated with 0.5 ml of Ellman's reagent. (19.8 mg of 5, 5-dithio-bis-2nitrobenzoic acid (DTNB) in 100 ml of 0.1 % sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). Then 0.4 ml distilled water was added, mixture was thoroughly mixed and absorbance was read at 412 nm and expressed as units/ml.

Estimation of Superoxide dismutase (SOD) activity

This was done using a standard procedure (Xin *et al*., 1991). One gramme of liver was collected form each rat in the different groups, blended and homogenized using from 37.37 IU/L to 44.54 IU/L, 50.25 IU/L normal saline. The homogenate was centrifuged and the supernatant was decanted. Superoxide dismutase levels in the supernatant was assayed.

Estimation of Catalase activity Catalase activity was measured according to the method of Aebi (1984). A given volume (0.1 ml) of the serum was pipetted into cuvette containing 1.9 ml of 50 mM phosphate buffer of pH 7.0. Reaction was started by the addition of 1.0 ml of freshly prepared 30 % v/v hydrogen peroxide. The rate of decomposition of hydrogen peroxide was measured spectrophotometrically from changes in absorbance at 240 nm. The enzyme activity was expressed as unit's ml/protein.

Lipid peroxidation: Malondialdehyde assay The lipid peroxidation was measured by determining the concentration of MDA in the blood according to the method of Ohkawa et al. (1979). Malondialdehyde was measured spectrophotometrically using the red fluorescent light that it gives out just like any other thiobarbituric reactive substance (Nair *et al.*, 2008).

Statistical Analysis

The results obtained from this study were analyzed using the Statistical Package for Social Sciences (SPSS) version 17.0 for Windows. Analysis of variance (ANOVA) was used to compare means, and values were considered significant at $P < 0.05$. Post Hoc multiple comparisons for differences between groups within groups were established using least significant difference (LSD). Results are presented as Mean ± S.E.M.

RESULTS AND DISCUSSION.

Effect of MSEE on glutathione activity of rats intoxicated with MSG is presented in Figure 1. Rats given only MSEE had glutathione level of 63.08±3.98 IU/L. However, MSG significantly ($P < 0.05$) reduced it to 37.37±2.27 IU/L. This reduction was ($P < 0.05$) significantly reversed by all doses of MSEE, 200, 400 and 800 mg/kg which caused significant and dose dependent increases in level of glutathione in treated rats and 51.09 IU/L, respectively. Glutathione has a major role as a reductant in oxidationreduction processes, and also serves in detoxication and several other cellular functions of great importance (Griffith, 1980). The extract was able to counter the strong oxidative ability of MSG probably by providing reducing agents which help to regenerate the oxidized glutathione as seen in the levels of reduced glutathione in MSGintoxicated rats treated with MSEE (Fig. 1).

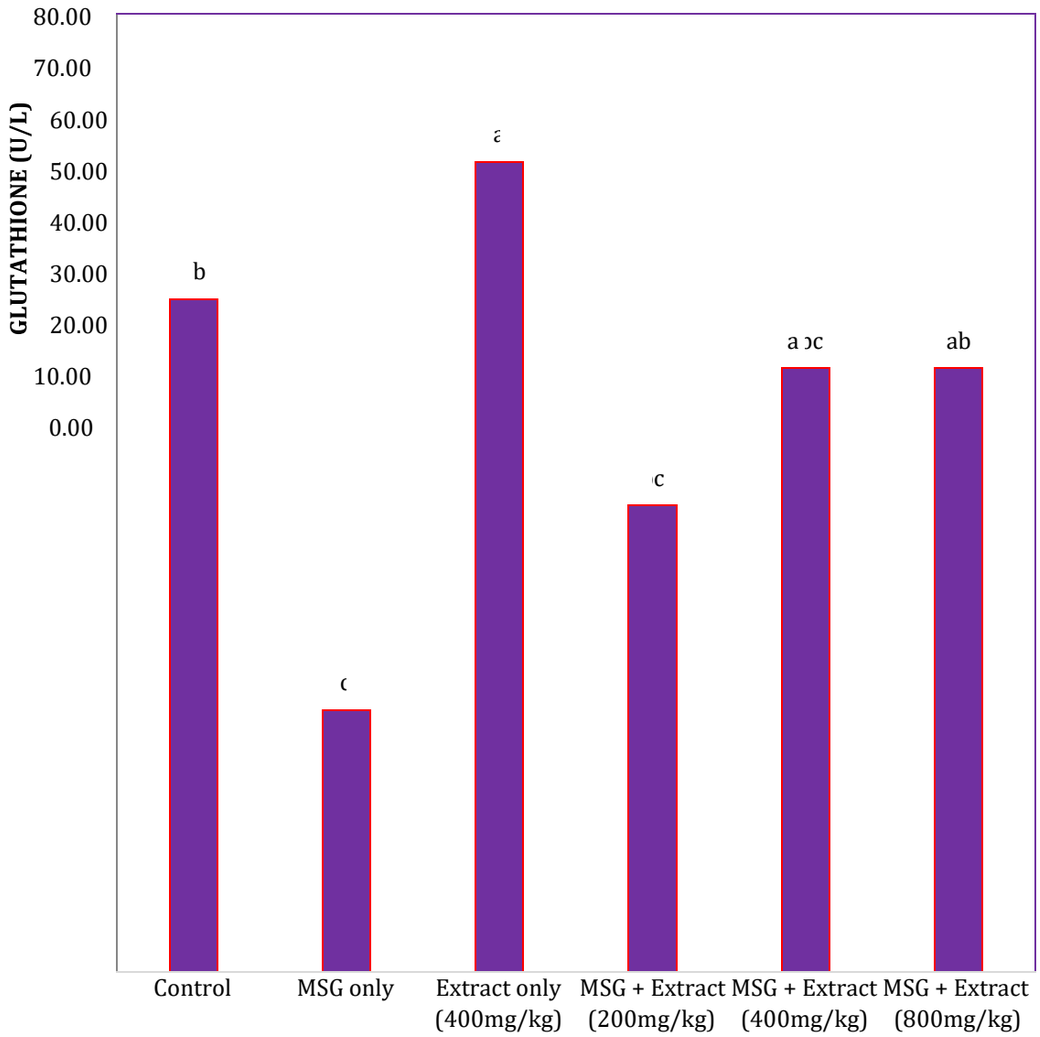

Fig 1: Glutathione level of rats intoxicated with MSG and treated with MSEE. Mean±SEM; Means with different superscript letters (a-d) are significantly different from each other (P<0.05)

Figure 2 shows the effect of MSEE on SOD activity of MSG-intoxicated rats. Rats intoxicated with MSG had a significant reduction in SOD from 26.28 IU/L in rats given only MSEE to 20.15 IU/L. The extract, at the dose of 400 mg/kg gave highest and significant ($P < 0.05$) reversal of the reductions in SOD of rats caused by MSG intoxication from 20.15 IU/L to 25.43 IU/L. Superoxide dismutase (SOD) an endogenous antioxidant enzyme has the ability to protect all the cells from superoxide radicals turning them to either hydrogen peroxide on reduction or to molecular oxygen on oxidation thereby preventing oxidative stress and other degenerative and inflammatory diseases arising from aerobic respiration by scavenging the superoxide radical which is the one electron reduced form of oxygen: $2O_2^-$ (Hayyan *et al.,* 2016). Superoxide Dismutase (SOD) has also been used to treat arthritis, prostate problems, corneal ulcers, burn injuries, inflammatory diseases, inflammatory bowel disease, and long-term damage from exposure to smoke and radiation, and to prevent side effects of cancer drugs (Seguí *et al.,* 2004). There was a depletion of SOD in MSG-intoxicated rats, as it counters the oxidative processes cause by MSG. This was however, reversed by MSEE which caused an increase in SOD activity.

Effect of MSEE on Catalase activity of MSG intoxicated rats is presented in Figure 3. Rats given only MSEE had catalase level of 31.00±1.39 IU/L while those intoxicated with MSG had catalase level of 23.75±0.73 IU/L ($P < 0.05$). This was significantly reversed by 800 mg/kg MSEE to 29.80 IU/L. Since catalase is an endogenous antioxidant enzyme that decomposes hydrogen peroxide to water and oxygen, thus which can lead to cellular damage, it

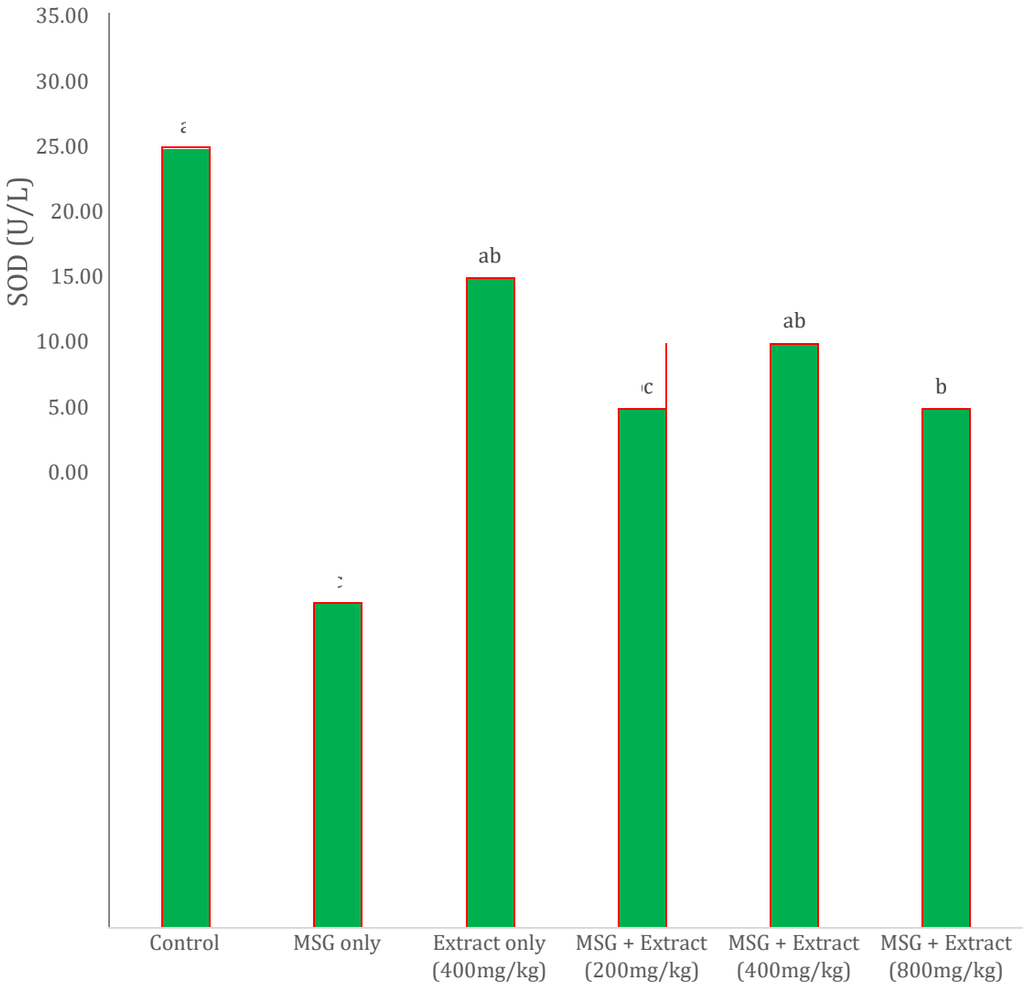

Fig 2: SOD level of all the groups. Mean±SEM; Means with different superscript letters (a-d) are significantly different from each other ($P<0.05$).

therefore means that MSG can cause cellular emergence of the malignant phenotype in damage. Catalase is mainly produced in the mouse keratinocytes among other conditions liver and sent to various parts of the body to (Nishikawa *et al*., 2002). It therefore shows protect the cellular organelles. Catalase also that the extract has the ability to eliminate removes H_2O_2 produced by SOD. Studies oxidative products of MSG especially at the have shown that a decrease in catalase dose of 800 mg/kg.
correlates with carcinogen-initiated

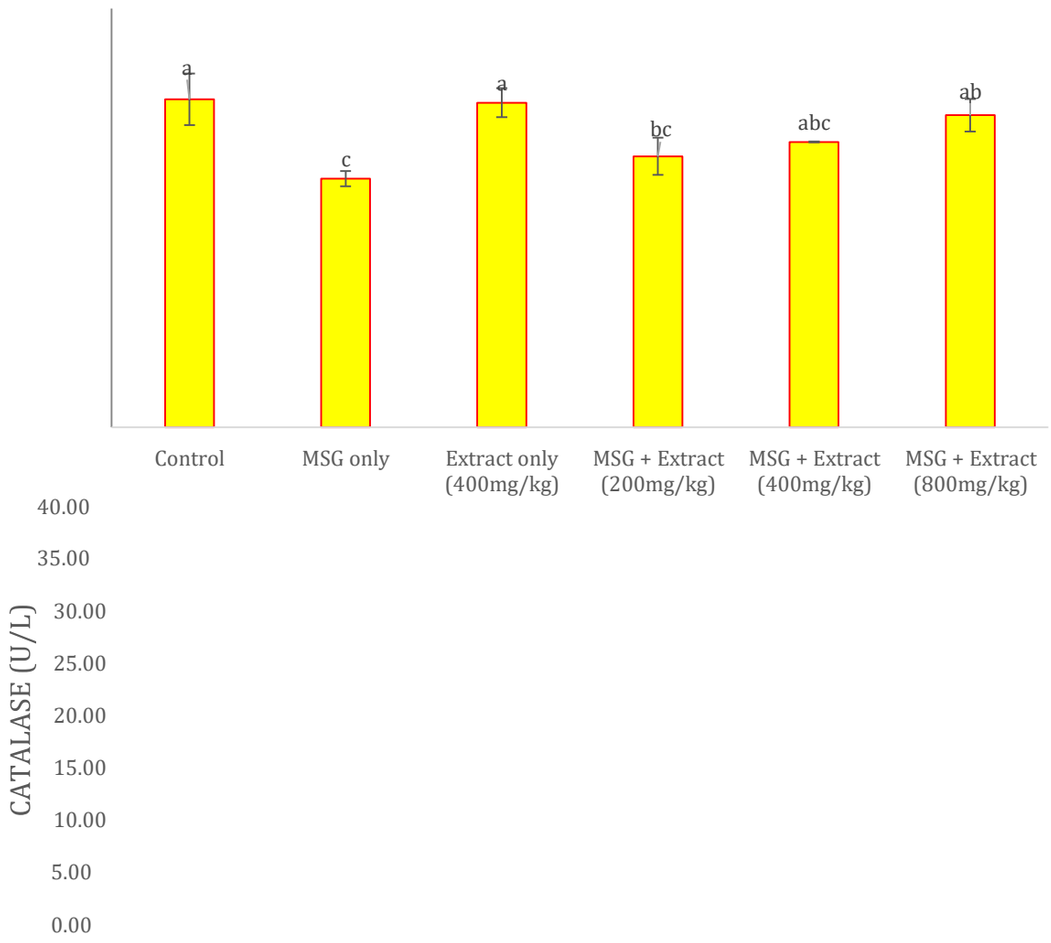

Fig 3: Effect of MSEE on catalase activity of MSG-intoxicated rats. Mean±SEM; Means with different superscript letters (a-d) are significantly different from each other (P<0.05)

Effect of MSEE on MDA levels of MSG-intoxicated rats is presented in Figure 4. The MDA level of the group intoxicated with MSG (8000 mg/kg) was significantly increased (3.88±0.24 mMol/L) when compared to the group given only MSEE at 400 mg/kg (1.18±0.06 mMol/L). The extract caused significant ($P < 0.05$) and dosedependent reductions sin the MDA levels of intoxicated rats, with the highest reduction in rats given MSEE 800 mg/kg which had MDA level of 2.17 mMol/L Lipid peroxidation has been established as a major mechanism of cellular injury in many biological systems of plant and animal origin and is measured by determining the concentration of MDA in the blood (Ohkawa *et al*., 1979). Malondialdehyde is a highly toxic by-product formed in part by lipid oxidation-derived free radicals, and its concentration increases with peroxidation, so it is a marker for oxidative stress (Davey *et al.*, 2005). The extract was able to significantly reduce MDA levels in MSG-intoxicated rats. This finding agrees with that of Farombi and Onyema (2016), who showed that the simultaneous administration of Vitamins C, and E and quercetin to MSG-treated rats significantly reduced the increase in MDA induced by MSG. Monosodium glutamate caused increase in MDA which was reversed by MSEE. This confirms the anti-oxidant activity of *M. sloanei* seeds in MSG intoxicated rats.

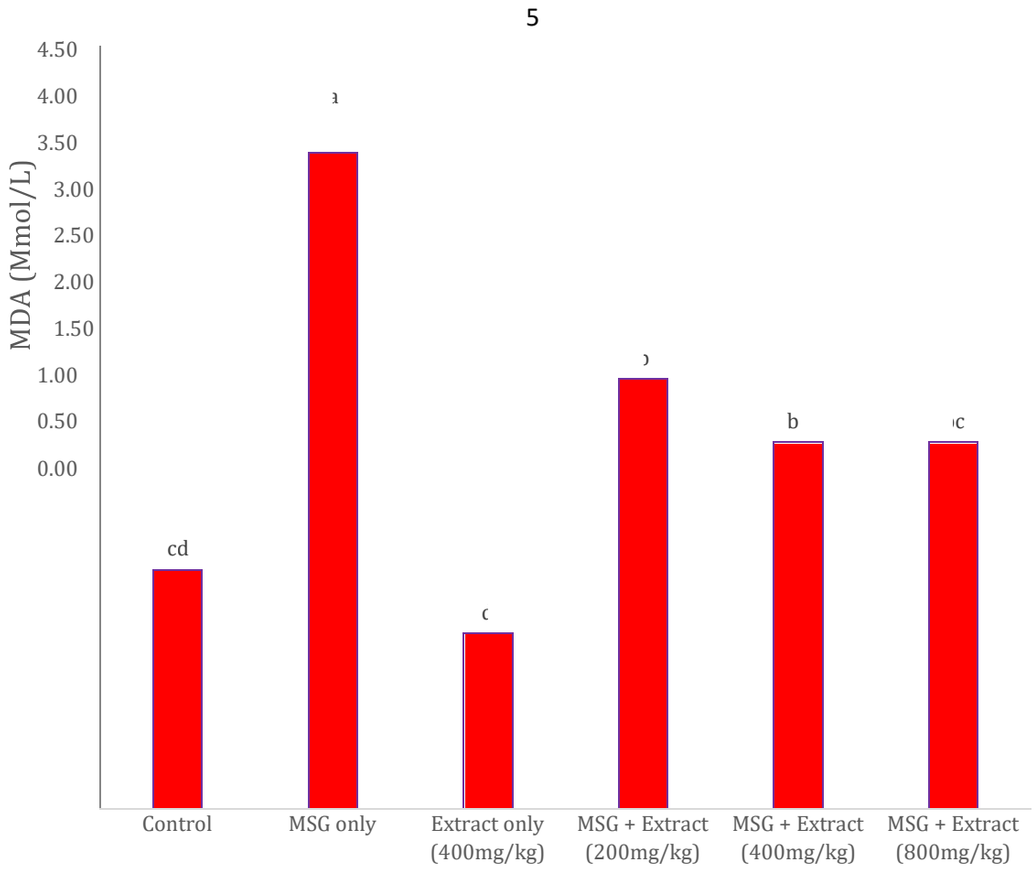

Fig 4: Effect of MSEE malondialdehyde levels of MS-intoxicated rats. Mean±SEM; Means with different superscript letters (a-d) are significantly different from each other (P<0.05)

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