ARACHIS HYPOGAEA SEED POWDER AMELIORATED 1, 2-DIMETHYLHYDRAZINE-INDUCED OXIDATIVE STRESS IN EXPOSED RATS

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ABSTRACT
This study evaluated the effect of Arachis hypogaea seeds on 1, 2 – dimethylhydrazine-induced oxidative stress in the colon and liver of male and female rats. Eighty-four rats of both sexes were used for this study and were divided into seven groups of 6 rats each. 1, 2- dimethylhydrazine (DMH) was administered subcutaneously at a dose of 25 mg/kg body weight. Group A (control) rats of both sexes were maintained on normal rat feed. The Group B rats were maintained on normal feed and administered DMH once weekly for 12 weeks. The Group C rats were provided normal feed and administered DMH weekly for 24 weeks. Group D rats were administered DMH and normal feed for 12 weeks followed by a peanut-supplemented diet for the next 12 weeks. The Group E rats received DMH weekly and peanut-supplemented diet concomitantly for 24 weeks. Group F rats had a peanut-supplemented diet for 12 weeks before DMH administration for another 12 weeks. Group G rats were maintained only on a peanut-supplemented diet for 24 weeks. The result shows that DMH significantly (p ≤ 0.05) increased malondialdehyde level in the colon but the increase was reversed by the incorporation of peanut in the diet. There were significant (p ≤ 0.05) decreases in antioxidant enzyme activities and reduced glutathione levels in the groups that received DMH alone which were reversed in the group of rats exposed to DMH and peanut-supplemented diet. The findings indicate that the consumption of peanut-supplemented diet reduced in rats, the increase in lipid peroxidation occasioned by DMH.

Keywords: Oxidative stress, Arachis hypogaea, 1, 2- dimethylhydrazine, colon carcinogenesis, antioxidant enzymes.

INTRODUCTION
The oxidation of polyunsaturated fatty acids (PUFAs) by reactive oxygen species (ROS) has the capacity to trigger the process of degradation of lipids, a biochemical event which leads to the release of free radicals and other harmful substances which include malondialdehyde (MDA), conjugated dienes, hydroperoxides, lipoperoxides, and toxic aldehydes (Cejas et al., 2004). Peroxidation of membrane PUFAs leads to alterations in cell membrane fluidity and alteration in the ability of the compromised cell to keep its constituents at equilibrium with its immediate extracellular medium. The compromised membrane structure also increases its porosity
Isoje, A.O. and Obi, F.O.: Arachis Hypogaea Seed Powder Ameliorated 1, 2-Dimethylhydrazine-Induced Oxidative Stress and inflammation (Finaud et al., 2006). Reactive oxygen species (ROS) which are notorious for causing changes in the activities of biomolecules are believed to be responsible for the onset of several pathological conditions (Valko et al., 2007; Perše, 2013). The generation of free radicals and their derivatives in excess amounts interfere with the structure and functions of diverse cellular macromolecules. Notable among the macromolecules are lipids, proteins, and DNA. ROS interactions with macromolecules modulate the expression of genes (Perše, 2013). The primary defense elements are enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR). These enzymes function collectively in defending the cell against ROS-induced biological changes. If the function of one enzyme is not accompanied by that of the others, lipoperoxidative membrane lipid damage remains certain (Al-Gubory et al., 2012, Deponte, 2013).

In our earlier study, we reported that peanuts contain several phytochemicals namely; tannins, saponins, flavonoids, alkaloids and coumarins. Further, we also found from in vitro antioxidant capacity studies that peanut extract had a better 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity relative to vitamin C, as evidenced by peanuts lower IC50 value of 3.04±0.11 µg mL−1 as against 8.01±0.42 µg mL−1 obtained for vitamin C (Isoje and Obi, 2021). It is therefore evident that peanuts may protect cells, tissues and organs against chemically-induced oxidative stress. Consequently, this study evaluated the effects of Arachis hypogaea seeds, sourced locally, in Nigeria (referred to as big red) on the activities of antioxidant enzymes and glutathione status in rats exposed to 1, 2-dimethylhydrazine.

**MATERIALS AND METHODS**

**Plant material**

*Arachis hypogaea* seeds were purchased at Uselu Market, Benin City. The verification of the peanut seed and plant was carried out by Dr. Henry A. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin. The peanut plant was then deposited at the herbarium and assigned a voucher number UBH_A352.

**Chemicals**

The following analytical grade reagents were used for this study;

1. 2- Dimethylhydrazine dihydrochloride (98%) (Sigma Aldrich, Germany), halothane (Piramal Healthcare Limited, India). All other chemicals not itemized were also of analytical grade.

**Animals**

A total of 84 healthy albino rats (Wistar strain) of both sexes were used for this study. They were purchased from the Department of Biochemistry Animal Unit, Faculty of Life Sciences, University of Benin, Benin City, Nigeria and housed in wood framed/ iron meshed cages in the same facility. The rats were acclimatized for 14 days prior to the beginning of the experiment. During the acclimatization period, they had unlimited access to water and feed (growers mash, product of Bendel Feeds and Flour Mills Limited, Ewu, Edo State, Nigeria).

**Rat diet formulation**

The peanut was incorporated into the rat diet at 20% level i.e. 20 g of peanut powder to 80 g of rat feed (Guyton et al., 2008).

**Preparation of 1, 2- dimethylhydrazine dihydrochloride (DMH) solution**

Stock DMH (98%) was dissolved in 1 mM ethylenediaminetetraacetic acid (EDTA-disodium salt) - saline solution just before use at a pH of 6.5 which was adjusted with 1 M NaOH to ensure that the carcinogen is stable at the time of use.

**Animal groups and treatment**

The acclimatized rats were divided into 7 experimental groups of 6 male rats each and another 7 groups of 6 female rats each
(labeled A to G). DMH was administered at a dose of 25 mg/kg body weight subcutaneously. Members of each group were housed separately in a clean, disinfected cage in a room with a 12-hour light/dark cycle. The rats were maintained on the normal diet, growsers mash, and the peanut-supplemented normal diet described above and water ad libitium. Group A (control) rats of both sexes were maintained on normal feed (growers mash) and a weekly subcutaneous injection of the vehicle (EDTA in Saline). Group B rats were provided normal rat feed and subcutaneous injection of DMH 25 mg/kg body weight (Veceric and Cerar, 2004) once weekly for 12 consecutive weeks. Group C rats were placed on normal feed and injected DMH 25 mg/kg body weight once weekly for 24 weeks. Group D rats were administered DMH, 25 mg /kg body weight and provided normal feed for 12 weeks followed by the peanut-supplemented diet (at 20% level) for the next 12 weeks. Group E rats were injected 25 mg DMH/kg body weight and the peanut-supplemented diet (20% level) concomitantly for 24 weeks. Group F rats were placed on a peanut-supplemented diet for 12 weeks prior to the administration of 25 mg DMH/kg body weight for 12 weeks. Group G rats were placed on a 20% peanut-supplemented diet throughout the experimental period of 24 weeks but received once weekly injection of the vehicle (EDTA- saline). All groups were kept on their respective diet for 24 weeks.

Animal Sacrifice, Collection and Preparation of samples for analyses

The study was carried out with strict compliance with the ethics in Guidelines and Specification on Experimental Animal Care (Animal care and use program, 2011). Rats in each group were sedated with halothane. While under anesthesia the abdominal and thoracic regions were opened and blood was collected into plain sample tubes by heart puncture. The colon and liver were excised and homogenized by grinding 1 g portions in an ice-cold mortar with acid-washed sand in 5 mL physiological saline. Each homogenate was subjected to centrifugation at 3500 rpm for 5 minutes and a clear supernatant was obtained which was kept at -20°C until needed.

Biochemical Assays

Superoxide dismutase activity was assessed by the method of Misra and Fridovich (1972) which involved following the auto-oxidation of adrenaline to adrenochrome at 420 nm. Catalase activity was examined by the method of Cohen et al., (1970), in which the decomposition of hydrogen peroxide was monitored at 480 nm. Glutathione peroxidase activity was determined by measuring the production of purpurogallin from pyrogallol at 420 nm as described by Nyman (1959). The method of Tietz (1969) was used to determine the level of reduced glutathione. Reduced glutathione analysis was based on the ability of 5, 5 dithiobis (2- nitrobenzoic acid) (DTNB) to undergo a reduction process in the presence of reduced glutathione (GSH) resulting in the production of a yellow compound. The reduced chromogen is directly proportional to the concentration of GSH. Malondialdehyde levels were measured based on its reaction with 2- thiobarbituric acid as described by Buege and Aust (1978).

Data analysis

The results obtained from the biochemical assays were expressed as mean ± standard deviation (SD). In order to establish whether the mean values were statistically significantly different from each other, analysis of variance (ANOVA) was done using SPSS software (Version 21.0).To know which means have differences that are significantly different, LSD multiple range test was done by employing the same SPSS computer software. Values were considered significant at p ≤ 0.05.

RESULTS

Malondialdehyde and reduced glutathione levels as well as catalase, superoxide dismutase and glutathione peroxidase activities in the colon and liver of treated rats
are presented in Tables 1 and 2. The results show a significant increase in MDA level in the group of rats treated with DMH alone relative to the control. Consumption of peanut-supplemented diet significantly reduced the MDA levels. Also, as shown in Table 1 there were corresponding decreases in the activities of the antioxidant enzymes in the groups that were administered with DMH only for 12 and 24 weeks but a corresponding elevation in their activities in rats that were administered with DMH and provided peanut-containing feed.

Table 1: Colon MDA and GSH levels and SOD, Catalase and GPx Activities of Male and Female Rats.

<table>
<thead>
<tr>
<th>MDA Level (nmol/g tissue)</th>
<th>SOD Activity (U/mg tissue)</th>
<th>Catalase Activity (U/mg tissue)</th>
<th>GPx Activity (Units/mg tissue)</th>
<th>GSH level (µmol/L, colon homogenate supernatant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Rats (Male)</td>
<td>Rats (Female)</td>
</tr>
<tr>
<td>A Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B DMH12/NC</td>
<td></td>
<td></td>
<td>2.30 ± 0.65</td>
<td>2.23 ± 0.04</td>
</tr>
<tr>
<td>C DMH12/NC</td>
<td></td>
<td></td>
<td>7.8 ± 0.87</td>
<td>7.9 ± 0.31</td>
</tr>
<tr>
<td>D DMH12/PNT12</td>
<td></td>
<td></td>
<td>3.31 ± 0.23</td>
<td>3.00 ± 0.07</td>
</tr>
<tr>
<td>E DMH12/PNT12</td>
<td></td>
<td></td>
<td>4.35 ± 0.28</td>
<td>4.15 ± 0.04</td>
</tr>
<tr>
<td>F PNT12/DMH24</td>
<td></td>
<td></td>
<td>1.40 ± 0.12</td>
<td>1.31 ± 0.06</td>
</tr>
<tr>
<td>G PNT12</td>
<td></td>
<td></td>
<td>4.15 ± 0.18</td>
<td>4.10 ± 0.06</td>
</tr>
</tbody>
</table>

*Control = Normal rat chow (NC) and water with weekly subcutaneous injection of EDTA-saline solution.

PNT = Peanut (Arachis hypogaea)

DMH12/Normal chow = Maintained on normal chow (NC) while treating with 1, 2-dimethylhydrazine (DMH) for 12 weeks. DMH24/Normal chow = Maintained on normal chow but treated with 1, 2-dimethylhydrazine (DMH) for 24 weeks. DMH12/PNT12 = Treated with DMH for 12 weeks while on normal rat chow and later maintained on peanut diet (PNT) for 12 weeks. DMH24+PNT24 = Treated with DMH and peanut simultaneously for 12 weeks. PNT12/DMH12 = Maintained on peanut diet for 12 weeks, followed with DMH treatment for 12 weeks. PNT24 = Maintained on peanut diet for 24 weeks with weekly subcutaneous injection of EDTA-Saline solution.

**Values with superscripts a, b, c, d, e or f are significantly different from the value of the group with the corresponding upper case letter A, B, C, D, E or F (p ≤ 0.05).
**DISCUSSION**

In this study, DMH-induced oxidative stress as evidenced by the increase in the malondialdehyde levels of the DMH-treated groups of rats relative to the control. The fact that DMH induced oxidative stress was also supported by the decreased status of antioxidant molecules and enzymes in the colons of rats of both sexes (Table 1). However, the provision of peanut-incorporated diet to the rats either pre-treatment, post-treatment or simultaneously with DMH caused a reversal in DMH-induced increases in malondialdehyde levels, decreased GSH levels, as well as in decreased activities of antioxidant enzymes. Jrah-Harzallah *et al.* (2013) reported similar increases in MDA levels in colon of rats exposed to DMH. Incorporation of peanuts in the diet consumed by rats before the administration of DMH caused male rats’ colon MDA levels to remain at values close to those of the control. This may be due to the action of the polyphenols contained in the peanuts (Isoje and Obi, 2021). However, the mechanism by which the polyphenols brought about these ameliorations remains substantially unclear at present. Polyphenols are however believed to have the capacity to enhance the detoxification of xenobiotics (Maru *et al.*, 2014) and thus decreasing their deleterious effects (Maru *et al.*, 2014). Again polyphenols are usually able to act as antioxidant molecules and enzymes in the colon (Maru *et al.*, 2014). Again, polyphenols are usually able to act as proton donors and so are ready sources of abstractable protons in place of membrane-

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**Values with superscripts a, b, c, d, e or f are significantly different from the value of the group with the corresponding upper case letter A, B, C, D, E or F (p \( \leq 0.05 \)).
bound polyunsaturated fatty acids. As such free radical-induced membrane damage and associated malondialdehyde release are reduced. This could be the cause of the attendant low MDA levels observed in rats exposed to DMH and peanut-supplemented diet. Malondialdehyde levels in the tissues are indicative of oxidative stress. The results obtained from this study agree with earlier reports of Eboh et al. (2015) and Ghareeb et al. (2018) that 1,2 dimethylhydrazine induces oxidative stress as evidenced in the present study by a rise in MDA levels in the colon of DMH exposed rats. The general lowering of MDA level in rats that received a 20% peanut diet indicates that the peanuts have anti-oxidative potency. Data from the present study showed a considerable depletion in all the antioxidant enzyme activities as well as in the level of the non-enzyme antioxidant molecule investigated in the group of rats exposed to DMH alone when compared to the results of the group treated with DMH and peanut-incorporated diet. So, the presence of peanut in the diet caused an elevation in the activities of antioxidant enzymes and in the level of the antioxidant molecules in the colon, liver and serum of rats of both sexes. Ray et al., (2000) and Okolie et al., (2013) opined that one of cancer cell's self-defense is the reduction of the antioxidant system and this appears to be a characteristic finding in pre-malignant cells. In agreement with this theory, the result of the present study showed depletion in the activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) and reduced glutathione levels. Eboh et al. (2015) and Ghareeb et al. (2018) reported a decline in the activities of antioxidants enzymes (GPx, GSH, catalase and SOD) in the colon and liver of rats administered DMH. Administration of DMH has been reported to induce the generation of toxic compounds in the colon and levels of such toxic metabolites are known to be reduced considerably by antioxidants (Hamiza et al., 2012). Supplementation with peanuts, before, during and after the administration of DMH significantly improved and restored colon and liver SOD activities to normal. This is more in consonance with what was reported earlier by Reid et al. (2016) that feeding peanuts to rats at 5% and 10% levels increased SOD activity significantly in azoxymethane-induced precancerous lesions in rats.

In conclusion, it is evident that the incorporation of peanuts in the diet provided to rats effectively reduced the DMH-induced oxidative stress in the animal colon as well as the liver. This protective action is not unconnected with the phytochemicals present in peanut many of which are polyphenols with antioxidant potential.

REFERENCES


