Assessment of histo-reparative effect of methanolic extract of *Camellia sinensis* in rat model of Lead-induced testicular damage

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**ABSTRACT**

**Objective:** To assess reparative effect of methanolic extract of *Camellia sinensis* on testicular histomorphology of male Wistar rats exposed to lead-induced testicular damage. **Methods:** Twenty four male Wistar rats were grouped into four A–D (n = 6). These include normal control group A (distilled water 5 ml/kg), test control group B (lead acetate 30 mg/kg), treatment group C (lead acetate 30 mg/kg + MECS 5 mg/kg) and treatment group D (lead acetate 30 mg/kg + MECS 10 mg/kg). Administrations were through oral route for a period of 60 days and body weight of study animals was recorded during days 0, 20, 40 and 60 of study. Phytochemical analysis of extract was done using GCMS. At the end of study, testicular tissue weight of study animals was measured followed by histopathological study. **Results:** The extract significantly ameliorated body weight loss and exhibited histo-reparative effect on testicular histomorphology of study animals following lead exposure. **Conclusion:** Methanolic extract of *Camellia sinensis* exhibits reparative effect against toxic effect of tissue toxicant due to its antioxidant activity.

**Keywords:** *Camellia sinensis*, Lead toxicity, Testis histomorphology, Wistar rats

**Article Info:** Received 03 June 2020; Review Completed 09 July 2020; Accepted 17 July 2020; Available online 15 August 2020

**Cite this article as:** Omotoso DR, Akinola AO, Ehiemere WP. Assessment of histo-reparative effect of methanolic extract of *Camellia sinensis* in rat model of Lead-induced testicular damage. *Journal of Drug Delivery and Therapeutics.* 2020; 10(4-s):46-52

http://dx.doi.org/10.22270/jddt.v10i4-s.4007

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**INTRODUCTION**

Medicinal plants are plant species containing phytochemical compounds, in some or all of its parts, which possess therapeutic properties that can be harnessed for therapeutic purposes1-3. Among the widespread natural biodiversity of medicinal plants is the *Camellia sinensis* (*C. sinensis*) which is an annual plant of Indian nativity but now globally distributed especially in the tropical regions with several of its parts applied in folkloric medicine for variety of therapeutic purposes4. It is commercially available in the form of green tea, a common herbal beverage that has been reported to exhibit variety of therapeutic properties5.

Basically, the medicinal value of medicinal plants is a function of therapeutic properties of their constituent bioactive or phytochemical compounds which in turn exert diverse physiological effects on body tissues and systems6-7. In essence, phytochemical screening provides a veritable means of assessing phytochemical profile of medicinal plants including *C. sinensis* in order to determine their therapeutic potential8. Different methods or techniques have been employed to phytochemically screen medicinal plant extracts or herbal preparations. One of such is the gas chromatography-mass spectrometer (GC-MS) technique which is an efficient separation technique that has been employed in profiling phytochemical constituents of medicinal plants including very-volatile compounds9,10.

The therapeutic effects of phytochemicals include protective or reparative effects that they exert against damaging effects of tissue toxicants especially heavy metals like lead. Exposure to lead has been associated with pathologies of different tissues such as brain, liver, kidney, spleen, bone marrow and testis following their lead-induced oxidative damage11-15. The mechanism of lead-induced tissue pathologies usually involve a shift of equilibrium between production or activation and removal or inactivation of oxidative radicals (such as superoxide radicals, hydrogen peroxide and hydroxyl radicals) towards an increased production or activation of the radicals leading to oxidative damage of tissues16,17. Therefore, potential therapeutic agent with antioxidant activity like *C. sinensis* may provide cytoprotection against deleterious effect of lead exposure. Some other therapeutic
activities associated with *C. sinensis* include anti-inflammatory activity, anti-cancer or anti-tumor activity, anti-diabetic activity and anti-microbial activity. In this study, the objective was to assess the reparative effect of methanolic extract of *Camellia sinensis* (MECS) on testicular histomorphology of male Wistar rats following exposure to lead-induced testicular damage.

### MATERIALS AND METHODS

#### Chemical reagents and MECS

Chemical reagents used in this study were procured from Bristol Scientific Co. Ltd. (Lagos, Nigeria). In addition, Green tea beverage produced in China was procured locally from Steven Chukw Global Associate Ltd. (Lagos, Nigeria) and was used to prepare MECS according to method by Khai et al.

#### GC-MS phytochemical analysis of MECS

0.1 g of MECS was dissolved in 1ml of methanol, filtered and 1 μl of the filtrate used for GC-MS phytochemical analysis of the extract. The GCMS analysis was performed using gas chromatograph (7890A series) coupled to TSQ quantum XLS mass spectrometer with an injector device (7683B series). The temperature and pressure of column heater were set at 250°C and 10.15 psi respectively, average velocity was 66.45 cm/sec and carrier gas was Helium (99.99%) with 1 ml/min flow rate. Electron impact (EI) of MS was set at 70 eV, injection volume was 1 μl, scan interval set at 0.5 seconds, scan mass range from 50 – 650 amu and polarity was positive. The phytochemical constituents of MECS were separated on a column TG-5ms (with dimensions ~ 30 μL, 0.25 mmID X 0.25 μm film thickness) and flame ionization detector (at 250°C) used to determine percentage composition of phytochemical compounds. The total running time of GC-MS was 30 minutes. The interpretation of mass-spectrum for identification of phytochemical compounds present in MECS was done by comparing with reference spectra in the database of National Institute of Standard and Technology (NIST) library.

#### Study animals

This study involved twenty four male Wistar rats with body weight range of 165-185 g. The study animals were housed at Animal House Facility, Igbinedion University Okada, Edos-State, Nigeria, where the study was also conducted. Within the Facility, the study animals were kept in animal cages at room temperature (24 ± 2°C), relative humidity of 50-55% and exposed to 12/12 hour light/dark cycle under hygienic condition. The animals were fed on standard animal feed and allowed unrestricted access to drinking water *ad libitum*.

#### Study design

The study animals were randomly divided into four groups (A-D) with each group comprised of six animals (*n* = 6). Group A animals represented normal control group administered with distilled water (5 ml/kg body weight (b.w.)); Group B animals represented test control group administered with lead acetate (30 mg/kg b.w. of 1% solution); Group C animals represented first treatment group administered with lead acetate (30 mg/kg b.w. of 1% solution) + MECS (5 mg/kg b.w.) while Group D animals represented second treatment group administered with lead acetate (30 mg/kg b.w. of 1% solution) + MECS (10 mg/kg b.w.). Dosages employed in this study were considered safe without toxic effects. All administrations were through oral route using a flexible oro gastric gavage coupled to calibrated hypodermic syringe and the period of administration was 60 days. During the administration period, body weight of study animals was measured and recorded during 0, 20, 40 and 60 days of study.

#### Tissue processing

At the end of administration period, study animals were sacrificed with the right and left testes harvested, weighed and processed. The average tissue weight of right and left testes was calculated for study animals in control and treatment groups. The tissue processing involved fixation in 10% Neutral Buffered Formalin, dehydration using ascending grades of alcohol (involving two changes of 70%, 90% and absolute alcohol), clearing in xylene, embedding in molten paraffin and cooling to form tissue blocks.

#### Tissue sectioning and staining

Blocks of study tissue were cut into 5 μ thick sections and stained using Haematoxylin and Eosin (H & E) technique. The staining protocol was as follows: dewax in xylene, hydrate with descending grades of alcohol (100%, 90% and 70% alcohol) and distilled water, stain in Haematoxylin, wash in running water, differentiate in 1% acid alcohol (1% HCl in 70% alcohol) for, blue in Schöl’s tap water for, rinse in distilled water, stain in Eosin, rinse in distilled water, dehydrate with ascending grades of alcohol (70%, 90% and 100% alcohol), clear in xylene and mount with Distrene polystyrene xylene (DPX).

#### Histopathological study

Stained tissue sections were examined under microscope to assess testis histomorphology of study animals in control and treatment groups. Photomicrographs of tissue sections were produced using digital camera for microscope, testicular histomorphological changes between control and treatment groups were comparatively assessed.

#### Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (IBM-SPSS, version 20) and results presented as Mean ± standard error of mean (SEM). Comparison of statistical results was done using one-way analysis of variance (ANOVA) and p<0.05 taken as significantly different level.

#### RESULTS

**Phytochemical analysis of MECS using GC-MS**

The GC-MS analysis of MECS revealed the retention time (Minutes) and peak area (%) of phytochemical compounds present in the extract as given in Table 1. The retention time represented relative concentration of compounds being eluted while peak area represented relative concentration of phytochemicals present in the extract. The gas chromatogram showed 16 peaks which indicate phytochemical compounds identified in the MECS by GC-MS technique (Figure 1). These include Sulfuric acid, cyclohexylmethyl dodecyl ester (0.45%), 1H-Pyrazole-4-carboxylic acid (0.43%), Caffeine (36.07%), 9,12-Octadecadienoic acid, methyl ester (0.86%), 9,12,15-Octadecatrien-1-ol (1.98%), Pythol (1.36%), Heptadecanoic acid, 10-methyl-, methyl ester (0.31%), Naphth[2,3-ij]pyrrole, decahydropyran-2,4-dione, 2,4-Pyridinedimethanol (4.27%), 3,4-Dimethoxyphenylacetone (24.33%), 3,4-Dimethoxy-N,N-dimethylaniline (10.84%), 2,3'-O-p-Anisidinyl-1,3-dimethoxyphenyl)-methyl]- (15.83%).
### Table 1: Phytochemical compounds identified in MECS by GC-MS technique

<table>
<thead>
<tr>
<th>S/N</th>
<th>Retention Time (Minutes)</th>
<th>Name of Compound</th>
<th>Peak Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.821</td>
<td>Sulfurous acid, cyclohexylmethyl dodecyl ester</td>
<td>0.45</td>
</tr>
<tr>
<td>2</td>
<td>15.730</td>
<td>1H-Pyrazole-4-carboxylic acid</td>
<td>0.43</td>
</tr>
<tr>
<td>3</td>
<td>16.521</td>
<td>Caffeine</td>
<td>30.14</td>
</tr>
<tr>
<td>4</td>
<td>16.754</td>
<td>Caffeine</td>
<td>3.64</td>
</tr>
<tr>
<td>5</td>
<td>16.924</td>
<td>Caffeine</td>
<td>2.22</td>
</tr>
<tr>
<td>6</td>
<td>17.034</td>
<td>Caffeine</td>
<td>0.07</td>
</tr>
<tr>
<td>7</td>
<td>19.017</td>
<td>9,12-Octadecadienoic acid, methyl ester</td>
<td>0.86</td>
</tr>
<tr>
<td>8</td>
<td>19.103</td>
<td>9,12,15-Octadecatrien-1-ol</td>
<td>1.98</td>
</tr>
<tr>
<td>9</td>
<td>19.262</td>
<td>Phytol</td>
<td>1.36</td>
</tr>
<tr>
<td>10</td>
<td>19.384</td>
<td>Heptadecanoic acid, 10-methyl-, methyl ester</td>
<td>0.31</td>
</tr>
<tr>
<td>11</td>
<td>19.893</td>
<td>Naphth[2,3-b]oxirene, decahydro-</td>
<td>0.21</td>
</tr>
<tr>
<td>12</td>
<td>23.939</td>
<td>3,4-Pyridinedimethanol</td>
<td>4.27</td>
</tr>
<tr>
<td>13</td>
<td>25.787</td>
<td>3,4-Dimethoxyphenylacetone</td>
<td>24.33</td>
</tr>
<tr>
<td>14</td>
<td>25.867</td>
<td>3,4-Dimethoxy-N,N-dimethylbenzylamine</td>
<td>10.84</td>
</tr>
<tr>
<td>15</td>
<td>26.212</td>
<td>2',3'-O-p-Anisylideneguanosine</td>
<td>3.06</td>
</tr>
<tr>
<td>16</td>
<td>26.882</td>
<td>1,2,4-Oxadiazole, 3-(1,3-benzodioxol-5-yl)-5-[[3,4-dimethoxyphenyl]methyl]-</td>
<td>15.83</td>
</tr>
</tbody>
</table>

**Figure 1:** Gas chromatogram of MECS using GC-MS
Evaluation of body weight of study animals

The mean values of body weight of study animals in groups A – D measured during days 0, 20, 40 and 60 of study were shown in Figure 2. As the study progressed, normal control group A animals showed gradual body weight increase however, exposure to lead causes significant body weight loss in test control group B animals while non-significant body weight reduction was observed among study animals in treatment groups C and D.

![Figure 2: Mean values of body weight of study animals in groups A-D recorded during days 0, 20, 40 and 60 of study. Group A = distilled water, Group B = lead acetate, Group C = lead acetate + 5 mg/kg MECS, Group D = lead acetate + 10 mg/kg MECS. (*,+ indicate significant difference from groups A and B at p<0.05 respectively).](image)

Evaluation of study tissue weight in study animals

The mean values of right testis, left testis and average testis weight of study animals in normal control group A, test control group B, and treatment groups C and D recorded at the end of study period were shown in Figure 3. The result showed that relative to normal control group A animals, exposure to lead causes significant (p<0.05) study tissue weight loss in test control group B animals while only non-significant study tissue weight reduction was observed among animals in treatment groups C and D.

![Figure 3: Mean values of right testis, left testis and average testis weight of study animals in groups A-D recorded at the end of study period. Group A = distilled water, Group B = lead acetate, Group C = lead acetate + 5 mg/kg MECS, Group D = lead acetate + 10 mg/kg MECS. (*,+ indicate significant difference from groups A and B at p<0.05 respectively).](image)
Histopathological study

The histological profile of testicular tissues of study animals in groups A-D (Figure 4) showed normal histomorphology in group A normal control animals but histopathological changes, including degeneration and atrophy of seminiferous tubules, were prominent in group B animals due to damaging effect of lead exposure. However, testicular histomorphology of groups C and D comprising lead-induced animals treated with MECS showed comparison with the normal histomorphology of group A animals indicating potent histo-reparative effect of MECS against damaging effect of lead exposure.

**Figure 4**: Representative photomicrographs of testis showing testicular histomorphology of study animals in groups A-D (H&E X 40). Group A = distilled water, Group B = lead acetate, Group C = lead acetate + 5 mg/kg MECS, Group D = lead acetate + 10 mg/kg MECS.

DISCUSSION

The medicinal value of plants has been described as a function of therapeutic properties of their constituent phytochemical compounds and this underscore the need for efficient phytochemical screening of medicinal plant extracts or herbal preparations. In several studies, GC-MS has been employed in identification, characterization and quantification of distinct phytochemical compounds present in medicinal plant extracts or herbal preparations. Based on results of this study (Table 1 and Figure 1), the phytochemical screening showed that MECS contain phenolic compounds which usually have characteristic antioxidant properties.

Lead exposure caused tissue pathologies due to its potency as tissue toxicant and such exposure often resulted into structural degeneration, morphological distortions and physiological dysfunctions in different body tissues which include the testis. According to results of this study, exposure to lead caused significant reduction in body weight of test control animals during the period of study. However, study animals treated with MECS showed non-significant body weight loss that characterized lead exposure (Figure 2). Similarly, test control animals showed significant testicular weight reduction while the tissue weight loss observed among treated animals was non-significant in comparison with the normal control animals (Figure 3).

These outcomes are in conformity with previous studies wherein the widespread deleterious effect of lead exposure on several body tissues resulted into a cumulative adverse influence on total body weight following significant reduction in weight of distinct tissues. Generally, testicular tissue mass is largely composed of differentiated and undifferentiated germ cells as well as interstitial cells of Leydig hence, significant reduction of testicular mass often follows oxidative destruction these components.

As earlier posited, therapeutic agent with antioxidant property such as *C. sinensis* may exhibit cytoprotective or reparative effect against damaging effect of lead exposure. In other words, the cytoprotective or reparative effect of *C. sinensis* can be associated with its antioxidant activity or free-radical scavenging property which, like its other therapeutic activities, is a function of its phytochemical constituents. Such cytoprotective or reparative effect may occur in form of amelioration or reparation of histomorphology of tissues following exposure to tissue toxicant.
According to results in this study, testicular histomorphology of test control animals showed prominent histopathological changes which include degeneration or atrophy of seminiferous tubules and widening of interstitial space compared to normal histomorphology of normal control animals (Figure 4). These histopathological changes are characteristic features of damaging effects of testicular tissue toxicants. However, testicular histomorphology of study animals treated with MECS showed relative comparison with normal control animals (Figure 4). This implied protective and reparative effect of the green tea extract against the damaging effect of lead exposure.

Previous studies had established antioxidant activity of green tea as the hallmark of its therapeutic properties and linked it to its abundant phenolic constituents. In particular, El-Shahat et al. had reported that protective effect of green tea extract against testicular histopathological changes induced by cadmium (another potently toxic heavy metal) was due to the anti-oxidant property of its constituent polyphenols such as catechin. The findings of their study affirmed the green tea as potent anti-oxidant agent that can normalize metabolic disorders involving testicular tissues.

CONCLUSION

This study underscores the potency of methanolic extract of C. sinensis as a cytoprotective or histo-reparative agent against the damaging effect of common environmental toxicant - lead on testicular histomorphology of Wistar rats and this can be veritably associated with the antioxidant property of phytochemical constituents of the extract.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

ACKNOWLEDGEMENT

The authors would like to appreciate the technical assistance of Mr Adediji of Central Animal House Facility, Igbinedion University, Okada, Edo State, Nigeria during this research study.

SOURCE OF FUNDING

This study was solely funded by the authors.

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