Antioxidative Effects of Allium Sativum Methanolic Extracts against Paracetamol induced Liver Toxicity

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ABSTRACT
This study was undertaken to investigate the antioxidative effects of Allium sativum methanolic extracts on paracetamol induced hepatotoxic rats. Fifty-four adult male albino rats comprising of nine normal and forty-five paracetamol hepatotoxic rats were used for the study. The different biochemical parameters assessed were determined before the start of the study and subsequently monthly for the duration of the study. The activities of antioxidant enzymes such as superoxide dismutase SOD, catalase CAT, reduced glutathione GSH as well as the concentration of malondialdehyde as an indicator of lipid peroxidation were measured to evaluate oxidative stress in the experimental rats. Blood samples were collected through the eye of the rats and serum was obtained by centrifugation and stored at -20oC prior to analysis. The effects of increasing dosages (200, 300 and 450mg/kg bw) of Allium sativum methanolic extracts studied produced a duration and dose dependent significant (p < 0.05) rise in superoxide dismutase, a duration and dose dependent significant reduction in malondialdehyde levels but a duration and dose independent significant rise in catalase and reduced glutathione of hepatotoxic rats when compared with those of the paracetamol, normal and silymarin control rats. The result of this experimental study provides sufficient scientific baseline information for the potential use of the methanolic extract of A. sativum as an antioxidant agent in the fight against oxidative stress mediated diseases. It may be concluded that A. sativum extracts may prevent oxidative changes in liver by reducing reactive oxygen species.

Keywords: Allium sativum, paracetamol, antioxidative effects, superoxide dismutase, catalase, reduced glutathione, malondialdehyde

INTRODUCTION
The pathogenesis of drug induced liver damage involves free radicals—mediated peroxidations of biological membrane as in all diseases, biological membranes are particularly prone to the adverse effects of reactive oxygen species [1,2]. This peroxidation of unsaturated fatty acids in biological membranes lead to a decrease of membrane fluidity, disruption of membrane integrity and function, which is paramount in the pathogenesis of many oxidative stress mediated diseases. Oxidative stress is an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage [3]. Under normal conditions, the physiologically important intracellular levels of reactive oxygen species (ROS) are maintained at low levels by various enzyme systems in the body but at high concentrations, they produce adverse modifications to cell components such as lipids, proteins and DNA [3]. In humans, oxidative stress is believed to be involved in the development of cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, fragile X syndrome, Sickle Cell Disease, lichen planus, vitiligo, autism, infection, Chronic fatigue syndrome and Depression [3]. Various tissues have different susceptibilities to oxidative stress [4] and the brain is particularly more vulnerable to oxidative damage due to relatively low levels of antioxidants, high levels of polyunsaturated fatty acids and oxygen utilization [5,6]. Antioxidants can act by preventing free radical formation, scavenging free radicals, facilitating the repair caused by free radicals or by favoring the antioxidant defense in the body [7]. Plant sources like turmeric, clove, grape seeds, apple, ginkgobiloba and fruit peels like lemon, orange are believed to have strong antioxidants. Antioxidants are beneficial in preventing disease complexes such as cardiovascular diseases, diabetes, cancer, rheumatoid arthritis, inflammatory bowel disease, pancreatitis, haematological and neurodegenerative diseases [8]. However, since this protection of body antioxidants may not be complete, when the formation of ROS is excessive, additional protective mechanisms of dietary antioxidants, which may

be found in herbs like A. sativum (garlic) will be of great importance. It is known that phytochemicals confer pharmacological relevance on medicinal plants generally and this is the cause of the growing research interest in herbal medicine. In view of the above, this present study was designed to determine the antioxidative effects of increasing dosage of Allium sativum (garlic) methanolic extracts against paracetamol induced liver toxicity in rats viz. superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA).

MATERIALS AND METHODS

Plant Materials

Allium sativum used for this study was bought from the Ogbete main market, Enugu, Enugu state, Nigeria. The plants were identified to species level [9] at the Herbarium Unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Animal Model

Fifty-four (54) adult white wistar strain male albino rats (R. norvegicus) weighing 180 to 200g were used for the study. They were fed ad libitum with 18% crude protein (Guinea feed) commercial feed and allowed to acclimatize for two weeks under standard photoperiodic condition in a clean rat cage with three rats per cage in the Post graduate research laboratory, University of Nigeria, Nsukka. All animals were maintained under the standard laboratory condition for temperature (26 ± 2°C), humidity (50 ± 5%) and light (12 hours day length) and were allowed free access to food and water.

Preparation of Extracts

Fresh healthy Allium sativum were washed, cut into small pieces and homogenized in a warring blender. The resulting mixture was soaked in two litres of 80% methanol. The mixture was allowed to stand for twenty four hours with intermittent shaking. Following filtration, the filtrate obtained was concentrated to dryness at 40°C using a rotary evaporator under reduced pressure. The dried extracts were weighed and then stored in a refrigerator.

Induction of Paracetamol Liver Toxicity in Rats

Paracetamol liver toxicity was induced by single administration of solution of paracetamol at 750mg/kg intraperitoneally. After 4 days only rats with ALT levels above 65U/l were considered hepatotoxic and used for the study.

Experimental Design

This study was carried out on paracetamol- induced hepatotoxic rats for twelve weeks. The experimental design was the three by three Latin square design. Fifty-four rats used were divided into two major groups:

Group I: Nine non-hepatotoxic rats (Normal control)

Group II: Forty-five paracetamol induced hepatotoxic rats.

The group I rats were three rats each in three different cages and each received 1ml/kg of 5% methanol solution daily throughout the duration of the study. The Group II rats (paracetamol induced hepatotoxic rats) were divided into three subgroups (IIa, IIb, IIc). The subgroup IIa was the paracetamol control, three rats in a cage, and was replicated thrice and had 3 rats each which received 750mg/kg of paracetamol only. Subgroup IIb was divided into 3 replicates (IIb1, IIb2, and IIb3) respectively each replicate had 3 rats and received 200 mg/kg, 300 mg/kg or 450 mg/kg of Allium sativum extracts orally daily. The subgroup IIc, three rats each in a cage and replicated thrice received the standard drug silymarin at 100mg/kg [10]. The biochemical parameters superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA) assessed were determined first before the start of the experiment and subsequently monthly for the duration of the study. Blood samples were collected from the rat through the retro-orbital plexus monthly for analysis. Serum was obtained by centrifugation (5000rpm for 10 mins) and stored at -20°C prior to analysis.

Evaluation of Biochemical Parameters

Serum thiobarbituric acid reactive substances was measured indirectly by measuring serum malondialdehyde an end product of unsaturated fatty acid peroxidation, which can react with

thiobarbituric acid to form coloured complex thiobarbituric acid reactive substances. Lipid peroxidation was measured by the method of [11] and expressed in mmol/ml. Catalase activity was measured by the method of [12]. 0.1ml of serum was added to cuvette containing 1.9ml of 50Mm phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0ml of freshly prepared 30mM H₂O₂. The rate of decomposition of H₂O₂ was measured spectrophotometrically from changes in absorbance at 240nm. Activity of catalase was expressed as U/l. To measure the reduced glutathione level, the serum in 0.1M phosphate buffer pH 7.4 was taken. The procedure was followed initially as described by [13]. The serum was added with equal volume of 20% trichloroacetic acid containing 1mM EDTA to precipitate the serum protein. The mixture was allowed to stand for 5 minutes prior to centrifugation for 10min at 200rpm. The supernatant 200µl was then transferred to a new set of test tubes and 1.8ml of the Ellman’s reagent (5, 5’ –dithiobis-2-nitrobenzoic acid) (0.1mM) was prepared in 0.3M phosphate buffer with 1% of sodium citrate solution. Then all the test tubes make up to the volume of 2ml. After completion of the total reaction, solutions were measured at 412nm against blank. Absorbance values were compared with a standard curve generated from standard curve from known GSH. SOD activity of the serum was analysed by the method described by [14]. Assay mixture contained 0.1ml of sample, 1.2ml of sodium pyrophosphate buffer (pH 8.3. 0.052M), 0.1ml phenazine methosulphate (180µM), 0.3ml of 300µM nitroblue tetrAzolium, 0.2ml NADH (750µM). Reaction was started by addition of NADH. After incubation at 30°C for 90s, the reaction was stopped by addition of 0.1ml glacial acetic acid. Reaction mixture was stirred vigorously with 4.0ml of n-butanol. Mixture was allowed to stand for 10 minutes, centrifuged and butanol layer was separated. Colour intensity of the chromogen in the butanol layer was measured at 560nm spectrophotometrically and concentration of SOD was expressed in U/l.

**Data Analysis**

The data collected was pooled and analyzed for their central tendencies using descriptive statistic, values were given as mean ± standard deviation of the observations. Analysis of variance and LSD was employed to test the significant differences (P < 0.05) among treatment means. All analyses were performed using SPSS for windows statistical software package version 20. The resulting outputs were presented in tables.

**RESULTS**

**Effects of Allium sativum Extracts on Superoxide Dismutase Level of Rats**

The effects of the increasing dosages (200, 300 and 450mg/kg) of *A. sativum* extracts produced a duration dependent and dose dependent, significant (p < 0.05) rise in the superoxide dismutase level of paracetamol hepatotoxic rats after the duration of treatment when compared with those of the paracetamol and silymarin control rats. Superoxide dismutase level were significantly lowered in paracetamol control groups throughout the duration of the study compared to all other treatment groups and also significantly lowered in all groups at the same period compared to the normal group. *A. sativum* at 200mg/kg raised superoxide dismutase level by 44.12%, at 300mg/kg it raised it by 48.53% while at 450mg/kg it raised it by 56.84% after the duration of treatments compared with paracetamol control at week 4 (Table 1). Silymarin at 100mg/kg raised superoxide dismutase level by 64.21% after the duration of treatment compared with paracetamol control at week 4 (Table 1). Normal control had no significant effect on superoxide dismutase level whereas the paracetamol treated control lowered superoxide dismutase level by 27.37%.

**Table 1. Effects of Allium sativum extracts on superoxide dismutase level of rats**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dosage</th>
<th>Week 0</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Week 12</th>
<th>% Change After 12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME</td>
<td>1.0ml/kg</td>
<td>16.91± 2.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.88± 2.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.60± 2.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.91± 2.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>PARA</td>
<td>750mg/kg</td>
<td>17.04± 2.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.50± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.60± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.90± 0.21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-27.37&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>AS</td>
<td>200mg/kg</td>
<td>17.23± 1.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.82± 0.43&lt;sup&gt;e&lt;/sup&gt;</td>
<td>13.18± 0.16&lt;sup&gt;i&lt;/sup&gt;</td>
<td>13.69± 0.64&lt;sup&gt;d&lt;/sup&gt;</td>
<td>44.12&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>300mg/kg</td>
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<td>13.40± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.77± 0.29&lt;sup&gt;j&lt;/sup&gt;</td>
<td>14.11± 0.19&lt;sup&gt;e&lt;/sup&gt;</td>
<td>48.53&lt;sup&gt;i&lt;/sup&gt;</td>
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<tr>
<td>AS</td>
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<td>SL</td>
<td>100mg/kg</td>
<td>16.94± 2.32&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>15.20± 0.14&lt;sup&gt;i&lt;/sup&gt;</td>
<td>15.60 ±0.37&lt;sup&gt;j&lt;/sup&gt;</td>
<td>64.21&lt;sup&gt;i&lt;/sup&gt;</td>
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</table>

Values given represents the Mean ± SD of 9 observations, mean values labeled with the same number superscripts (1 – 4) along the same row are not significantly different at 5% significance level (p < 0.05). Mean values labeled with the same alphabets superscripts (a – l) on the same column are not significantly different at 5% significance level (p < 0.05). ME = 5% Methanol solution represents the Non- hepatotoxic control, PARA = Paracetamol negative control, AS = Allium sativum, SL=Silymarin representing hepatotoxic control. % change = % change from para treated at weeks 4 compared to all treatments groups at week 12. Negative % change denotes decrease; Positive % change denotes an increase.

Effects of Allium sativum Extracts on Catalase Level of Rats

The effects of the increasing dosages (200, 300 and 450mg/kg) of A. sativum methanolic extracts produced a duration dependent and dose independent, significant (p < 0.05) changes in the catalase level of paracetamol hepatotoxic rats after the duration of treatment when compared with those of the paracetamol and silymarin control rats. Catalase level were significantly lower in paracetamol control groups throughout the duration of the study compared to all other treatment groups whereas it was significantly higher in all groups at the same period compared to the normal group. A. sativum at 200mg/kg raised catalase level by 10.14% at 300mg/kg it raised it by 3.49% while at 450mg/kg it reduced it by 3.17% after the duration of treatments compared with paracetamol control at week 4 (Table 2). Silymarin at 100mg/kg raised catalase level by 0.61% after the duration of treatment compared with paracetamol control at week 4 (Table 2). Normal control had no significant effect on catalase level whereas the paracetamol treated control lowered catalase level by 26.92%.

Table2. Effects of Allium sativum extracts on catalase level of rats.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dosage</th>
<th>Week 0</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Week 12</th>
<th>% Change After 12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME</td>
<td>1.0ml/kg</td>
<td>134.11± 6.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134.22± 5.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134.00±5.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>135.00±5.76&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td>PARA</td>
<td>750mg/kg</td>
<td>135.56± 6.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.44±5.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.00±3.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.67±5.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-26.92</td>
</tr>
<tr>
<td>AS</td>
<td>200mg/kg</td>
<td>135.33± 6.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.67±2.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>84.89±3.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80.89±1.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.14</td>
</tr>
<tr>
<td>AS</td>
<td>300mg/kg</td>
<td>135.67± 5.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86.67±7.43&lt;sup&gt;d&lt;/sup&gt;</td>
<td>78.67±2.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>76.00±2.74&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.49</td>
</tr>
<tr>
<td>AS</td>
<td>450mg/kg</td>
<td>135.56± 5.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>75.00±3.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.11±4.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.11±4.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-3.17</td>
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<tr>
<td>SL</td>
<td>100mg/kg</td>
<td>134.56± 5.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.67±3.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80.78±5.87&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.61</td>
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</table>

Values given represents the Mean ± SD of 9 observations, mean values labeled with the same number superscripts (1 – 4) along the same row are not significantly different at 5% significance level (p < 0.05). Mean values labeled with the same alphabets superscripts (a – l) on the same column are not significantly different at 5% significance level (p < 0.05). ME = 5% Methanol solution represents the Non- hepatotoxic control, PARA = Paracetamol negative control, AS = Allium sativum and SL=Silymarin representing hepatotoxic control. % change = % change from para treated at weeks 4 compared to all treatments groups at week 12. Negative % change denotes decrease; Positive % change denotes an increase.

Effects of Allium sativum Extracts on Reduced Glutathione Level of Rats

The effects of the increasing dosages (200, 300 and 450mg/kg) of A. sativum methanolic extracts produced a duration dependent and dose independent, significant (p < 0.05) rise in the reduced glutathione levels of paracetamol hepatotoxic rats after the duration of treatment when compared with those of the paracetamol and silymarin control rats. Reduced glutathione level were significantly lower in paracetamol control groups throughout the duration of the study compared to all other treatment groups and also it was significantly lower in all groups at the same period compared to the normal groups. A. sativum at 200mg/kg raised reduced glutathione levels by 58.18%, at 300mg/kg it raised it by 36.27% while at 450mg/kg it raised it by 85.33% after the duration of treatments when compared with paracetamol control at week 4 (Table 3). Normal control had no significant effect on reduced glutathione level whereas paracetamol treated control lowered reduced glutathione levels by 23.30%.

Table 3: Effects of Allium sativum extracts on reduced glutathione level of rats.

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<tr>
<th>Treatments</th>
<th>Dosage</th>
<th>Week 0</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Week 12</th>
<th>% Change After 12weeks</th>
</tr>
</thead>
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<tr>
<td>ME</td>
<td>1.0ml/kg</td>
<td>37.44 ± 2.44&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>37.13 ± 2.74&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>PARA</td>
<td>750mg/kg</td>
<td>37.41 ± 2.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.57 ± 1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.62 ± 1.72&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>12.71 ± 1.66&lt;sup&gt;e&lt;/sup&gt;</td>
<td>-23.30</td>
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<tr>
<td>AS</td>
<td>200mg/kg</td>
<td>37.16 ± 2.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.16 ± 1.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.08 ± 1.42&lt;sup&gt;c,d&lt;/sup&gt;</td>
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<td>15.21 ± 3.07&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>AS</td>
<td>450mg/kg</td>
<td>37.76 ± 2.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.91 ± 3.57&lt;sup&gt;j&lt;/sup&gt;</td>
<td>25.21 ± 1.89&lt;sup&gt;k&lt;/sup&gt;</td>
<td>30.71 ± 2.02&lt;sup&gt;l&lt;/sup&gt;</td>
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<td>26.31</td>
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Values given represents the Mean ± SD of 9 observations, mean values labeled with the same number superscripts (1 – 4) along the same row are not significantly different at 5% significance level (p < 0.05). Mean values labeled with the same alphabets superscripts (a – l) on the same column are not significantly different at 5% significance level (p < 0.05). ME = 5% Methanol solution represents the Non- hepatotoxic control, PARA = Paracetamol negative control, AS = Allium sativum, SL= Silymarin representing hepatotoxic control. % change = % change from para treated at week 12. Negative % change denotes decrease, Positive % change denotes an increase.

Effects of Allium sativum Extracts on Malondialdehyde Level of Rats

The effects of the increasing dosages (200, 300 and 450mg/kg) of A. sativum methanolic extracts produced a duration dependent and dose dependent significant (p < 0.05) reductions in the malondialdehyde level of paracetamol hepatotoxic rats after the duration of treatment when compared with those of the paracetamol and silymarin control rats. Malondialdehyde level were significantly higher in paracetamol control group throughout the duration of the study compared to all other treatment groups whereas it was significantly higher in all groups at the same period compared to the normal group. A. sativum at 200mg/kg reduced malondialdehyde level by 57.11%, at 300mg/kg it reduced it by 60.57% while at 450mg/kg it reduced it by 68.80% after the duration of treatments compared with paracetamol control at week 4 (Table 4). Silymarin at 100mg/kg reduced malondialdehyde level by 71.77% after the duration of treatment compared with paracetamol control at week 4 (Table 4). Normal control had no significant effect on malondialdehyde level whereas the paracetamol treated control raised malondialdehyde level by 45.82 %.

Table 4: Effects of Allium sativum extracts on malondialdehyde level of rats.

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<thead>
<tr>
<th>Treatments</th>
<th>Dosage</th>
<th>Week 0</th>
<th>Week 4</th>
<th>Week 8</th>
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<th>% Change After 12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME</td>
<td>1.0ml/kg</td>
<td>6.11 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.12 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.04 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.18 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
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<tr>
<td>PARA</td>
<td>750mg/kg</td>
<td>6.10 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.69 ± 1.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.11 ± 3.09&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>16.17 ± 1.44&lt;sup&gt;c,d&lt;/sup&gt;</td>
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<td>AS</td>
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<td>15.22 ± 2.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.59 ± 2.60&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>AS</td>
<td>450mg/kg</td>
<td>6.08 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.39 ± 0.78&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10.94 ± 1.65&lt;sup&gt;h&lt;/sup&gt;</td>
<td>10.20 ± 2.79&lt;sup&gt;j,h&lt;/sup&gt;</td>
<td>-68.80</td>
</tr>
<tr>
<td>SL</td>
<td>100mg/kg</td>
<td>6.04 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.22 ± 1.74&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.19 ± 1.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.23 ± 2.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-71.77</td>
</tr>
</tbody>
</table>

Values given represents the Mean ± SD of 9 observations, mean values labeled with the same number superscripts (1 – 4) along the same row are not significantly different at 5% significance level (p < 0.05). Mean values labeled with the same alphabets superscripts (a – l) on the same column are not significantly different at 5% significance level (p < 0.05). ME = 5% Methanol solution represents the Non- hepatotoxic control, PARA = Paracetamol negative control, AS = Allium sativum and SL= Silymarin representing hepatotoxic control. % change = % change from para treated at weeks 4 compared to all treatments groups at week 12. Negative % change denotes decrease; Positive % change denotes an increase.

DISCUSSION

The oxidative damage in tissue can be limited by exogenous antioxidants and the antioxidant defense system of the host, the most important defenses are enzymatic antioxidants, such as SOD, CAT and non enzymatic antioxidant as GSH [15]. SOD is a manganese containing enzyme and is the first antioxidant enzyme to deal with oxy-radicals by accelerating the dismutation of superoxide to hydrogen peroxide while CAT is a peroxisomal heme protein that catalyses the removal of hydrogen peroxide formed during the reaction catalyzed by SOD [16]. GSH is a tripeptide (L-γ-glutamyl cysteinyI glycine), antioxidant and a powerful nucleophile, critical for cellular protection such as detoxification from reactive oxygen species and excretion of toxic molecules and control of the inflammatory cytokine cascade [17]. Depletion of GSH in tissue leads to impairment of the cellular defense against reactive oxygen species, and may result in peroxidative injury. In this study, these antioxidant parameters (SOD, CAT, GSH) were inactivated by lipid peroxides or free radicals, which may have resulted in decreased level of these enzymes in paracetamol control rats [18]. The levels of these enzymes in the serum were significantly decreased in paracetamol hepatotoxic rats compared to the control rats suggesting impaired antioxidative defense, impaired functions and damage of liver. Plant extracts administration to the paracetamol treated rats elevated the SOD, CAT and GSH level in the liver emphasizing the antioxidant activity of the plant extracts. Impaired detoxification systems was seen in paracetamol hepatotoxic rats, since serum GSH content and antioxidant enzymes (SOD, CAT) level were reduced. Similar results were obtained by [19] using carbon tetrachloride, they detected a significant decrease in liver GSH content after carbon tetrachloride intoxication. The observed decrease in GSH level in paracetamol hepatotoxic rats might have been due to an increased scavenging of reactive substances that were produced as a result of the necrotic and/or steatic state of the hepatocytes and/ or possible decreased hepatic production of GSH [19]. Various enzymatic and non enzymatic systems have been developed by the cell to attenuate ROS, however, when a condition of oxidative stress is established, the defense capacities against ROS become insufficient. Therefore, ROS affects the antioxidant defense mechanisms, reduces the intracellular concentration of GSH, decreases the level of SOD and enhances lipid peroxidation [20]. In agreement with these explanations, the observed decrease in SOD recorded in paracetamol hepatotoxic rats may be due to inactivation of the antioxidative enzymes that may have been caused by increased accumulation of superoxide radicals, which could further stimulate lipid peroxidation. The antioxidative action observed by plant extracts in this study is in accordance with previous reports suggesting that A. sativum is potent against reactive oxygen species-mediated damages [21,22]. It has also been reported that consumption of garlic enhances the intracellular contents of glutathione in all cells including those in normal liver and mammary tissue.

Lipid peroxidation has been implicated in the pathogenesis of various liver injuries and subsequent liver fibro-genesis in experimental animals and humans [23]. MDA is a major reactive aldehyde that appears during the peroxidation of biological membrane poly unsaturated fatty acid [24]. Therefore the hepatic content of MDA is used as an indicator of liver tissue damage involving a series of chain reactions [25]. The significant increase in the serum MDA concentration in this study indicated increased lipid peroxidation caused by administration of paracetamol. The significant dose dependent decrease in the serum MDA concentration confirms that pre-treatment with A. sativum extracts could effectively protect against the serum lipid peroxidation induced by paracetamol, this is in agreement with the work of [26]. The ability of paracetamol to induce lipid peroxidation of liver membrane lipids may be the basis of paracetamol liver toxicity. A correlation between the metabolism of paracetamol, lipid peroxidation and hepatocyte damage provides the strongest supporting evidence. Under some conditions, lipid peroxidation may determine the extent of injury by amplifying the injury through propagation of free radical processes, generating toxic compounds and impairing detoxification systems [27]. The exact mechanism of action of the plant extracts is not known in reducing lipid peroxidation but their antioxidant properties may have played a major role.

CONCLUSIONS

From the results of this experimental study, A. sativum showed potent antioxidative properties. It was evident that A. sativum extracts was able to significantly raise the antioxidant parameters due to paracetamol liver toxicity. Plant extracts have the ability to down regulate free radicals elevation and...
reduce oxidative stress induced by paracetamol liver toxicity. These encouraging results may have future clinical implications in oxidative stress mediated diseases because of the increased use of natural herbs worldwide and Nigeria in particular.

REFERENCES


