Biochemical and Histopathological Changes on the Effect of Oral Administration of Methanol Extract of *Allium Fistulosum* (Leaf and Bulbs) In Liver and Kidney in Thioacetamide-Induced Albino Rats

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**ABSTRACT**

The effect of methanol extract of the whole plant of *Allium fistulosum* was assessed for some biochemical and histological effects in albino rats. 25 male albino rats were used comprising of 5 groups and 5 rats per group. Rats in groups 3 and 4 were pretreated and post-treated, respectively, with methanol extract administered orally at 200 mg/kg body weight for 21 consecutive days while group 5 was administered 200 mg/kg vitamin C. Rats in groups 2, 3 and 5 were injected 400 mg/kg body weight of thioacetamide on the 21st day while group 4 was induced on 1st day. The Alanine aminotransferase (ALT) level increase significantly in untreated rat group 2 (24.16 ± 1.44) compared to normal control group 1 (20.46 ± 1.37). However, there was non-significant decrease in ALT levels of rats pretreated and post-treated with extract of *A.* fistulosum (19.80 ± 2.09 and 18.93 ± 0.38), respectively, compared to the normal control (P<0.05). There was no significant difference in Aspartate aminotransferase (AST) levels in the extract treated groups compared to normal control (group 1). Alkaline phosphatase (ALP) concentrations decrease significantly, in extract treated groups 3 and 4 (43.58 ± 2.79 and 41.14 ±1.26), respectively, compared to normal control (45.62 ± 2.22) (P<0.05). Urea concentration decrease significantly in extract post-treated group 4 (13.85 ± 0.46) compared to normal control (14.50 ± 0.34). However, the creatinine concentration decrease significantly in extract pretreated rat group 3 (13.50 ± 3.01) compared to the normal control (17.57 ± 0.08). Concentration of total protein also increase significantly, in rat groups 3 and 4 pretreated and post-treated with extract of *A. fistulosum* (67.2 ± 0.60 and 73.0 ± 0.17) compared to group1(58.8 ± 0.60) (P<0.05). There was no significant difference in the levels of total bilirubin in the extract treated groups compared to the normal control. However, total bilirubin level decrease significantly in extract treated groups compared to the untreated group. Mean weights of liver of animals in groups 3 and 4 (5.6±2.10 and 5.85±1.28) increase significantly, compared to normal control group 1 (4.68±1.63). There was no significant difference in the mean weights of kidney in group 3 and 4 (1.15±0.13 and 1.04±0.33), respectively, compared to the normal control group1 (1.09±0.7). The result hematoxylin and eosin (H and E) revealed that administration of *A. fistulosum* caused no noticeable defects in the histopathology of liver and kidney in extract treated groups compared to the controls.

**Key words:** *Allium fistulosum*, Creatinine, Total bilirubin and Thioacetamide.

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**INTRODUCTION**

Spring onion (*Allium fistulosum*) is perennial species that originated from Eastern Asia, of the family Liliaceae, genus *Allium* L. and of onion Species *A. fistulosum* L. (Vlase et al., 2013). The *Allium* genus includes biannual...
bulbous plants that grow in the north hemisphere with a few species found in South America and Tropical Africa (Salmiah et al., 2013). Other common names of the plant include; green onion, scallion, Welsh onion, Japanese bunching onion and salad onion. They may be referred to any young green onion stalk that is grown from spring onions, common onions, or other similar members of the genus Allium (Salgado et al., 2011). The spring onion, does not develop bulbs, and possesses hollow leaves (“fistulosum” means “hollow”) and scapes. In Wales, the spring onion has a dialectal variation, jibbons or sibwns which originates from the French ciboule (Fritsch, 2002). This plant is rich in two chemical groups that often provide benefits to human health; flavonoids and alk(en)yl cysteine sulfoxides. Allium species contain various organosulfoxides, particularly alk(en)yl cysteine sulfoxides, such as allicin, and γ-glutamylcysteines, some of which are responsible for their characteristic odor and flavour, as well as most of their biological properties (Vimal and Devaki, 2004; Vlase et al., 2013). Allium species have been used as ingredients in many dishes and with ethno medical purposes for many years. Medicinal properties have been attributed to it since ancient times, in addition to its culinary uses (fresh, cooked or dehydrated), which recently led to an accurate chemical analysis of its most characteristic active components. Onion and its organ sulfur constituents are studied extensively for their chemo preventive potential against cancer (Le Bon et al., 2000).

In a French epidemiological study, higher onion intake was correlated with lower risk of breast cancer (Sengupta et al., 2004). Compounds from onions have a range of health benefits such as antidiabetic, hypcholesterolemic, antithrombotic, anticarcinogenic, antiplatelet, antiasthmatic and fibrinolytic properties, and other various biological actions including antibiotic effects (Salgado et al., 2011). Onions are not only rich sources of core nutrients but are frequently eaten which make their nutrients valuable contribution to the diet and their phytochemical compounds that are of most interest nutritionally. Allium vegetables have been employed for a long time in traditional medical practice to treat a variety of diseases. Onion (Allium cepa L.), Allium vegetables, have been used for centuries for its pungency and flavoring value, and medicinal properties. The bulb of onion is used medicinally and has been consumed as seasoning food for many centuries (Sengupta et al., 2004). High intakes of spring onions have been directly associated with the management and prevention of obesity (Lee et al., 2008). Onion extract supplementation was shown to reduce the amounts of mesenteric fat and influenced the adipokine production at a transcriptional level in the high-fat induced obese animal model (Kim et al., 2012). Onion extracts reduced blood low-density lipoprotein cholesterol and increased high-density lipoprotein cholesterol of high-fat feeding Sprague-Dawley rats (Lee et al., 2012). Thioacetamide has been reported to cause hepatocellular necrosis, bridging necrosis and lymphocytic infiltrate without any cholestasis and based on the International Society for Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN) recommendations, a toxin model of hepatic encephalopathy was adopted using thioacetamide (Butterworth, 2011). The model was reported to be similar to human acutely progressive hepatic disorders with the parallel involvement of the brain (Butterworth et al., 2009). This model has been used to clarify changes in the functions of the liver in hepatic encephalopathy (Butterworth, 2011; Guerit, 2009). The present study was designed to evaluate the effect of oral administration of methanol extract of A. fistulosum on biochemical and histopathological changes in liver and kidney of rat induced-thioacetamide.

**MATERIALS AND METHODS**

**Experimental Animals**

25 male wistar rats of average weight between 140 to 170 g were used for the study. The animals were obtained from the Animal House of the College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State. The animals were moved to the Animal House in the Department of Medical Biochemistry. The animals were housed in stainless-steel cages under standard laboratory conditions of 27 ± 2°C, relative humidity 50 ± 15% and normal photo period (12 h dark/12 h light). Acclimatization took place for one week. The rats were grouped into 5 groups with 5 rats each. All animals received human care according to the criteria outlined in the guide for care and use of laboratory animals" prepared by the National Academy of science.

**Chemicals/Reagents**

The chemicals and reagents used include; thioacetamide and methanol are products of BDH chemical company limited (Poole, England), normal saline, distilled water. The commercial kits for AST, ALT, ALP, Bilirubin, Total Protein, Urinary creatinin, Albumin are products of Randox Laboratories Limited, United Kingdom.

**Medicinal Plant**

Spring onion (A. fistulosum) was obtained from Swali market in Yenegoa, Yenegoa Local Government Area, Bayelsa State, Nigeria. The plant was identified by Professor K. Ajibeshin in the Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University.

**Extraction and Preparation of Plant**

The plant (A. fistulosum) was washed and cut into tiny pieces. 2 kg of the plant material was then accurately
measured using a top loading balance and placed into a suitable container. The bulbs and leaves were then crushed using an electric blender. 5 liters of methanol was added to the container and this was kept for two days. The container was shaken morning and evening for this period of time for maceration to properly take place. The macerated sample was then filtered with sintered glass funnel and cotton wool to eliminate particles. The filtrate was then evaporated using a thermostat water bath at 45°C to evaporate the methanol. The viscous concentrate obtained weighed 80 g and kept in the refrigerator until it was needed.

**Experimental Procedure**

The experimental animals were divided into five groups comprising of five rats in each group and were treated as follows. GROUP 1: normal saline, GROUP 2: normal saline + 400 mg/Kg body weight of thioacetamide (i,p) on the 22nd day, GROUP 3: 200 mg/kg/day A. fistulosum administered orally for 21 days + 400 mg/Kg body weight of thioacetamide (i,p) on the 22nd day (Pretreated group), GROUP 4: 400 mg/kg thioacetamide (i,p) on day 1, fasted for 24 h + 200 mg/kg/day A. fistulosum administered orally for 20 days (Post treated group) and GROUP 5: Vitamin C (200 mg/kg body weight orally) for 21 days + 400 mg/Kg body weight thioacetamide. All groups were allowed access to feed and water freely for 21 days. They fasted for 24 h and sacrificed by cervical dislocation.

**Collection of Samples**

Blood samples were collected in plain bottles through the cardiac puncture and were centrifuged at 2,800 rpm for 10 min. Serum obtained was used for biochemical analysis.

**Biochemical Assay**

The following biochemical tests were carried out using various commercial assay kits from Randox Laboratory Limited, Company, Antrim, United Kingdom; AST, ALT, ALP, Total protein, Total bilirubin, Albumin, Creatinine and Urea.

**Histological Studies**

The abdominal cavity was opened up using a pair of forceps to expose the liver and kidney which were quickly dissected out and fixed in 10% formal saline for routine histological techniques. The tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 5 µm thick were obtained using a rotatory microtome. The deparaffinized sections were stained routinely with H and E method (Drury et al., 1967). Photomicrographs of the desired sections were made for further observations.

**Statistical Analysis**

The results were expressed as mean± SD. Data was analyzed by one-way analysis of variance. Sequential differences among means were calculated at the level of P<0.05, using Turkey contrast analysis as needed.

**RESULTS**

The effect of methanol extract of A. fistulosum in albino rats induced thioacetamide is presented. The results indicated significant decrease in mean weight of animals in groups 3 and 4 (9.19± 1.92 and 2.96±1.38) pretreated and post treated with methanol extract of A. fistulosum, respectively, compared to the normal group 1 (P<0.05). However, animals in group 5 showed slight weight loss (Table 1). Mean weights of liver of animals in groups 3 and 4 (5.6±2.10 and 5.85±1.28) increase significantly, compared to normal control group 1 (4.68±1.63). There was no significant difference in the mean weights of kidney in group 3 and 4 (1.15±0.13 and 1.04±0.33), respectively, compared to the normal control group1 (1.09±0.7) (Table 2). The ALT level increase significantly in untreated rat group 2 (24.16 ± 1.44) compared to normal control group 1 (20.46 ± 1.37). However, there was non-significant decrease in ALT levels of rats pretreated and post treated with extract of

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**Table 1. Mean weights of experimental rats on day 1 and day 21 of the study in grams.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial weight (g) Day 1</th>
<th>Final weight (g) Day 21</th>
<th>Difference in mean Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>149.47 ± 10.97</td>
<td>160.68 ± 12.23b</td>
<td>11.21 ± 1.26^a</td>
</tr>
<tr>
<td>2</td>
<td>144.12 ± 20.97</td>
<td>153.31 ± 19.05</td>
<td>9.19 ± 1.92^b</td>
</tr>
<tr>
<td>3</td>
<td>151.77 ± 20.04</td>
<td>154.73 ± 18.66</td>
<td>2.96 ± 1.38^c</td>
</tr>
<tr>
<td>4</td>
<td>165.79 ± 11.09</td>
<td>174.58 ± 11.54</td>
<td>8.79 ± 0.45^d</td>
</tr>
<tr>
<td>5</td>
<td>158.87 ± 27.21</td>
<td>158.03 ± 26.67</td>
<td>-0.84 ± 0.54^d</td>
</tr>
</tbody>
</table>

Values are means of five determinations± SD. Values with different superscript in the column differ significantly (P<0.05).
Table 2. Effect of *A. fistulosum* on mean weights of liver and kidney in thioacetamide-induced rats (grams).

<table>
<thead>
<tr>
<th>Organs</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (g)</td>
<td>4.68±1.63a</td>
<td>3.37±1.21b</td>
<td>5.6±2.10c</td>
<td>5.85±1.28c</td>
<td>5.59±1.64c</td>
</tr>
<tr>
<td>Kidney (g)</td>
<td>1.09±0.7a</td>
<td>0.84±0.24a</td>
<td>1.15±0.13a</td>
<td>1.04±0.33a</td>
<td>1.11±0.14a</td>
</tr>
</tbody>
</table>

Values are means of five determinations± SD. Values with different superscript in the row differ significantly (P<0.05).

Table 3. Effect of *A. fistulosum* on some biochemical indices in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>ALB (g/l)</th>
<th>UREA Mmol/l</th>
<th>Crea. µmol/dl</th>
<th>T.P (g/dl)</th>
<th>Total bil. µmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.46±1.37</td>
<td>20.72±0.73</td>
<td>45.62±2.22</td>
<td>49.24±2.67</td>
<td>14.50±0.34</td>
<td>17.57±0.08</td>
<td>58.8±0.60</td>
<td>7.94±0.50</td>
</tr>
<tr>
<td>2</td>
<td>24.16±1.44</td>
<td>21.52±1.01</td>
<td>46.18±1.71</td>
<td>50.14±1.77</td>
<td>14.26±0.40</td>
<td>17.55±0.17</td>
<td>63.0±0.78</td>
<td>9.32±0.69</td>
</tr>
<tr>
<td>3</td>
<td>19.80±2.09</td>
<td>20.57±0.55</td>
<td>43.58±2.79</td>
<td>50.20±0.97</td>
<td>14.58±0.23</td>
<td>13.50±3.01</td>
<td>67.2±0.60</td>
<td>8.74±0.50</td>
</tr>
<tr>
<td>4</td>
<td>18.93±0.38</td>
<td>20.57±0.53</td>
<td>41.14±1.26</td>
<td>47.23±0.59</td>
<td>13.85±0.46</td>
<td>16.58±0.05</td>
<td>73.0±0.17</td>
<td>8.03±0.50</td>
</tr>
<tr>
<td>5</td>
<td>23.92±1.72</td>
<td>20.91±1.01</td>
<td>45.25±1.32</td>
<td>48.28±1.74</td>
<td>17.08±0.96</td>
<td>17.32±0.53</td>
<td>73.2±0.35</td>
<td>9.36±0.57</td>
</tr>
</tbody>
</table>

Values are means of five determinations± SD. Values with different superscript in the column differ significantly (P<0.05).

*A. fistulosum* (19.80 ± 2.09 and 18.93 ± 0.38), respectively, compared to the normal control (P<0.05). (Table 3). There was no significant difference in AST levels in the extract treated groups compared to normal control (group 1). ALP concentrations decrease significantly, in extract treated groups 3 and 4 (43.58 ± 2.79 and 41.14 ± 1.26), respectively, compared to normal control (45.62 ± 2.22) (P<0.05) (Table 3). Urea concentration decrease significantly in extract post-treated group 4 (13.85 ± 0.46) compared to normal control (14.50 ± 0.34). However, the creatinine concentration decrease significantly in extract pretreated rat group 3 (13.50 ± 3.01) compared to the normal control (17.57 ± 0.08). Concentration of total protein also increase significantly, in rat groups 3 and 4 pretreated and post-treated with extract of *A. fistulosum* (67.2 ± 0.60 and 73.0 ± 0.17) compared to group 1 (58.8 ± 0.60) (P<0.05) (Table 3). There was no significant difference in the levels of total bilirubin in the extract treated groups compared to the normal control. However, total bilirubin level decrease significantly in extract treated groups compared to the untreated group. The effect of methanol extract of *A. fistulosum* on the histopathology of liver and kidney in animals induced thioacetamide is presented. The results of the study revealed that the extract pretreated group 3 displayed congestion of portal triad with normal hepatocytes. The photomicrograph of untreated group 2 showed loss of radial appearance, sinusoidal dilatation and normal hepatocytes. Group 4 and 5 displayed loss of tissue architecture while, the normal control group 1 showed normal hepatocytes, central vein, hepatic plate and endothelium lining the sinusoid (Figures 1 to 5). Histology of the kidney also, revealed that normal control group 1
Figure 2. Group 2: Loss of tissue architecture. H and E X 100.

Figure 3. Group 3: CPT congested portal triad; hepatocytes are normal H and E X 100.

Figure 4. Group 4: Red circle= loss of radial appearance and sinusoidal dilatation hepatocytes are normal H and E X100.
Figure 5. Group 5: loss of tissue architecture H and E X 100.

Figure 6. Group 1: kidney with normal architecture, G= glomerulus BS= bowman's space, RC= renal corpuscle, PCT= proximal convoluted tubule, DCT= distal convoluted tubule, SST= simple squamous epithelium lining the Bowman's capsule and SCE= simple cuboidal epithelium H and E X100.

displayed a normal glomerulus, Bowman’s space, renal corpuscle, proximal convoluted tubules, distal convoluted tubule, simple squamous epithelium lining the Bowman’s capsule and a simple cuboidal epithelium. The extract pretreated group 3 showed perforations in the stroma of the kidney while in extract post-treated group 4, there was no notable distortion of renal architecture when compared to the normal control. (Figures 6 to 10).

DISCUSSION
The present study was designed to evaluate the effect of oral administration of methanol extract of *A. fistulosum* on biochemical and histological tests in liver and kidney of rat induced-thioacetamide. Liver and kidney are two important organs that perform vital function for healthy survival of the body. Liver detoxify harmful substances, secretes bile into intestine, synthesizes and store important molecules, the kidney helps in maintaining homeostasis of the body by reabsorbing important material and excreting waste products. In the biochemical test, ALT level increase significantly in untreated rat group 2 compared to normal control group 1, which may be attributed to the toxic effect of the thioacetamide on
the animals Farjam et al. (2012) reported that acute hepatic encephalopathy can be induced in rats at a dose of 300 mg/kg/day for four days with increasing level of biomarker enzymes and severe pathological distortion. The Liver function test parameters ALT, AST, ALP and bilirubin are markers of various hepatic and cardiovascular conditions when elevated above certain concentration ranges (Rosen, 2000). Also, rise in level of ALT accompanied by elevation in the level of AST has been shown to play a role in the conversion of amino acid to keto acid and both AST and ALT are excellent markers of liver damage caused by exposure to toxic substances (Ranjna, 1999). However, rat groups 3 and 4 (pretreated and post-treated, respectively), with methanol extract of A. fistulosum showed a non-significant (p<0.05) reduction in the level of serum ALT (p<0.05) compared to the normal control group and a significant decrease in the serum ALT level in the extract treated groups compared to the untreated rats group 2. Analysis of chemical composition of A. fistulosum was reported as an excellent source of minerals, vitamins A, C and K, in addition to flavonoids, anthocyanins, saponins, sulfoxides and carotenoids (Fritsch et al., 2002).

The antioxidant property of this plant has been attributed to its numerous phytochemicals (Cuzzocrea and Caputi, 2001; Faraz and Amalinejad, 2003 Vlase et al., 2013). ALP concentrations decrease significantly, in extract treated groups 3 and 4 compared to normal control (P<0.05). Elevated ALP is often associated with biliary obstruction with cholestasis and usually before a rise in bilirubin (Friedman et al., 2003). Therefore, the reduced level of ALT and ALP in the extract
treated groups may be due to these phytochemicals that have been earlier reported as excellent antioxidant. The results also showed a significant increased (p<0.05) in serum total protein levels in rats pretreated and post treated with extract of A. fistulosum compared to the controls which could be an indication that the synthetic function of the liver was improved. A decrease in serum total proteins was earlier reported as indications of hepatotoxicity (Sallie et al., 1991). There was no significant (p<0.05) difference in the serum level of urea and creatinine except at 200 mg/kg body weight pretreated animal, which showed significant (p<0.05) reduction in the level of creatinine compared to the control. Urea is the main end product of protein catabolism. Deamination of amino acids takes place in the liver, through a cyclical process known as urea cycle. Ammonia is converted into urea and is excreted through urine which represents 90% of the total urinary nitrogen excretion. Urea varies directly with protein intake and inversely with the rate of excretion. Some of the urea is bound to heamoglobin so its concentration in red blood cells is greater than in the plasma. Renal diseases which diminish the glomerular filtration lead to urea retention and decrease in urea is seen in severe liver disease with destruction of cells leadings to impairment of the urea cycle (Ranjna, 1999). Creatinine is a waste product formed in muscle by creatinine metabolism. Creatinine is synthesized in the liver, passes into the circulation and is taken up by skeletal muscle. Its retention in the blood is evidence of kidney impairment.
The significant decrease in mean weight of animals in groups 3 and 4 (pretreated and post-treated), respectively, with methanol extract of *A. fistulosum*, compared to the normal group 1 is an indication that the extract may have weight lowering potential on the animals. This is in agreement with the earlier study carried out by some researchers on the anti-obesity activity of ethanol extract of *A. fistulosum* L. in high-fat induced obese mice and was reported that the extracts significantly reduced body weight, white adipose tissue weight and adipocyte size of the treated mice compared to high-fat induced control mice (Sung et al., 2011). This study shows that the extract has no effect on the liver and kidney of treated animals. The result H and E revealed that administration of *A. fistulosum* caused no noticeable defects in the histopathology of liver and kidney in extract treated groups compared to the controls. The study revealed that the extract pretreated group 3 displayed congestion of portal triad with normal hepatocytes. The photomicrograph of untreated group 2 showed loss of radial appearance, sinusoidal dilatation and normal hepatocytes which could be due to the toxicity effect of thioacetamide. Group 4 and 5 displayed loss of tissue architecture while, the normal control group 1 showed normal hepatocytes, central vein, hepatic plate and endothelium lining the sinusoid. Histology of the kidney also, revealed that normal control group 1 displayed a normal glomerulus, Bowman’s space, renal corpuscle, proximal convoluted tubules, distal convoluted tubule, simple squamous epithelium lining the Bowman’s capsule and a simple cuboidal epithelium. The extract pretreated group 3 showed perforations in the stoma of the kidney while in extract post-treated group 4, there was no notable distortion of renal architecture when compared to the normal control.

**CONCLUSION**

Methanol extract of *A. fistulosum* administered orally to pretreated and post-treated rats at a concentration of 200 mg/kg offer protection to the liver and kidney as reflected in the lowering effect on ALT and ALP, while increasing the level of total protein, which may be of chemically benefit to individuals at risk of hepatotoxic disease as well as nephrotoxic disease due to its non-significant (p<0.05) effect on creatinine and urea. Histopathology of the liver and kidney of treated groups shows no distortion compare with normal control.

**REFERENCES**


Fritsch RM, Friesen N (2002). Evolution and Taxonomy Domestication Pflanzen genetik Institut für und Kulturpflanzenforschung, D-06466 Gatersleben, Germany; Botanischer Garten der Universitä, D-49076 Osnabrück, Germany.


