Some biochemical parameters and histopathological features following prolonged administration of aqueous pod extract of *Acacia nilotica* in albino rats

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Accepted 17 January, 2012

The effects of aqueous pod extract of *Acacia nilotica* was investigated in rats. The pod extract of *A. nilotica* was obtained by Soxhlet extraction using distilled water as a solvent. The aqueous extract was administered for a period of 21 days at 50, 100 and 200 and 400 mg/kg body weight, respectively. The liver enzymes and some biochemical parameters were evaluated. The tissue samples were also taken for histopathological preparations. The effect of the extract significantly (P<0.05) increased values of Aspartate amino transferase (AST), Alanine aminotransferase (ALT) and Creatinine. While, significantly (P>0.05) decreased values of serum albumin, total protein, glucose and triglyceride at the end of the 21 days. While alkaline phosphatase (ALP) and Urea values were not affected. The histopathological study revealed marked haemorrhages of lung, heart and kidney with renal tubular degeneration. Liver revealed hepatocellular necrosis and mononuclear cell infiltrations, mild congestion of the stomach and mild sub mucosal mononuclear cell infiltrations of the small intestine, respectively. It was therefore concluded that *A. nilotica* extract has the potential to produce some level of toxicities and it consumption for medicinal purposes should be discourage.

Key words: Histopathological features, *A. nilotica* aqueous pod extract, biochemical parameters.

INTRODUCTION

*Acacia nilotica*: (English Names; thorn tree, wattles, Babul, Black babul, Indian Arabic gum), a member of the Family; *Fabaceae*. Tender pods and shoots are used as vegetables and are fed to camels, sheep and goats especially in Sudan, where it is said to improve milk production from these animals. In South Africa, the Zulu’s take the stem bark extract for cough and the Chipi1 use root bark for tuberculosis treatment, while the Masai use the stem bark and root decoction, to alleviate mood. In Ayurvedic medicine, the stem bark is considered a remedy for treating premature ejaculation (Pande et al., 1981). In Nigeria local traditional healers used the bark and pods extract for the treatment of ailments such diarrhoea and stomach ache. Although, the world health organization (WHO) in recognition of the increased value of herbal medicine to primary health care, has advocated for the proper identification, sustainable exploitation n, scientific development and appropriate utilization of herbal medicine which provide safe and effective remedies in Medicare (Wambebe, 1998). Many herbs including the decoction from the pods of *A. nilotica* have been used in folk medicine for the control of diarrhoea. *A. nilotica* although widely used in north eastern Nigeria for treatment purposes, it has not been scientifically evaluated for its use and toxicity. The scientific investigation of *Acacia nilotica* could support its reported efficacy in herbal medicine.

MATERIALS AND METHODS

Plant collection, identification and extract preparation

Fresh pods and leaves of *A. nilotica* were collected from Lai –Lai grazing reserve, Potiskum Local Government Area of Yobe State, Nigeria and submitted for confirmation to Dr. S.S Sanusi, Department of Biological Sciences, University of Maiduguri and a
voucher specimen was deposited at the Department of Veterinary Physiology and Pharmacology herbarium, University of Maiduguri, Nigeria. The pods were air dried at room temperature for three weeks. The crushing of the pods was done in laboratory using pestle and mortar, after which it was ground into powder. About (200 gm) of the powdered pod was weighed and introduced into a conical flask and 1 litre of distilled water was added thereafter. The mixtures was then shaken and allowed to stand for 30 min, after which it was boiled for 1 h, cooled and shaken vigorously, before filtration using whatman No. 1 filter paper. The filtrate was concentrated in a rotatory evaporator and stored at 4°C until used, and the yield was 6.75% (w/w).

Sub acute toxicity studies
Twenty five (25) Wister albino rats of both sexes (weighing between 140 to 160 g) were randomly selected and divided into five groups of five rats each, were used for the studies. Group I was used as control and Groups II, III, IV and V were treated orally with 50, 100 and 200 and 400 mg/kg of aqueous pod extract of A. nilotica, respectively for a period of 21 days. At the end of the treatment period, the rats were humanely sacrificed and blood samples collected to obtain serum following centrifugation. Serum samples harvested were used for the determination of the liver enzymes and biochemical parameters.

Estimation of liver enzymes and some biochemical parameters
ALT and AST were assayed using kits based on the method of Reitman and Frankel (1957). Alkaline phosphatase was determined using the method of Deutsche and Kelinsche (1972). The estimations of some biochemical parameters were also carried out using standard procedures according to manufacturer’s instructions.

Histopathology
The tissue samples of heart, lungs, small intestine, stomach, liver and kidney obtained were fixed in 10% formalin. The tissues were dehydrated through graded concentration of ethanol (70, 95% and absolute), cleared in xylene and embedded in paraffin wax. The embedded tissues were stained with hematoxylene and eosin (H and E) for light microscopic examination. The lesions observed were photographed using Vanox T. Olympus photographing microscope (Drury and Wallingthon, 1979).

Statistical analysis
All values were expressed as Mean±Standard Deviation. While analysis of variance (ANOVA) was used to analyse the extent of variation between groups and P values equal to or less than 0.05 were considered significant (Mead and Curnow, 1982). The computer soft ware Graph pad instat was used to analyse the data.

### RESULTS

#### Extraction

The aqueous pod extract was light green and have slight bitter taste. The yield was 6.75% (w/w).

#### Effect of prolonged oral administration of aqueous pod extract of A. nilotica on liver enzymes

The result of the effect of prolonged oral administration of A. nilotica water extract was presented in Table 1. Administration of the extract orally for 21days significantly (P<0.05) increased the levels of the liver enzymes (AST and ALT) in the treated rats when compared with the control. The levels of ALP were not affected by the extract treatments (Table 1).

#### Effect of prolonged administration of A. nilotica extract on some biochemical parameters

Total protein, albumin, cholesterol and triglyceride concentrations in the serum of extract treated rats were significantly (P<0.05) decreased when compared to the control (Table 2). Urea values was unaffected by the extract treatments, however, creatinine levels were significantly (P<0.05) increased in the treated animals when compared with the control group.

#### Histopathological studies

Treatment of rats with A. nilotica pod extract for 21 days resulted in the presence of lesions in the organs and tissues (Figures 1 to 6). Treatment of the rats with the extract at 400 mg/kg resulted in a mild congestion (Figure 1) of the mucous membrane of the stomach. Extract treatment at 200, and 400 mg/kg produced moderate sub mucosal mononuclear cell infiltrations of the intestine (Figure 2). The kidneys of rats treated with the extract showed marked congestion, tubular degeneration and

### Table 1. Effect of the aqueous pod of A. nilotica on mean liver enzymes.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>50 mg/kg</th>
<th>100 mg/kg</th>
<th>200 mg/kg</th>
<th>400 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (µ/L)</td>
<td>154.0±8.10</td>
<td>171.0±5.90</td>
<td>171.0±6.50</td>
<td>177.0±9.90</td>
<td>182.0±11.2</td>
</tr>
<tr>
<td>ALT (µ/L)</td>
<td>22.8±6.5</td>
<td>47.0±3.4*</td>
<td>54.0±7.4*</td>
<td>48.6±3.7*</td>
<td>52.0±8.9</td>
</tr>
<tr>
<td>ALP (µ/L)</td>
<td>177.0±16.7</td>
<td>172.0±47.0</td>
<td>170.0±36.0</td>
<td>161.0±15.0</td>
<td>192.0±9.4</td>
</tr>
</tbody>
</table>

b = Mean±Standard deviation based on five observations. *P<0.05 means significantly different from the control.
Table 2. Effect of the aqueous pod extract of *A. nilotica* on mean\(^{b}\) on some biochemical parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>50 mg/kg</th>
<th>100 mg/kg</th>
<th>200 mg/kg</th>
<th>400 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (mmol/L)</td>
<td>35.6±1.10</td>
<td>27.6±4.40*</td>
<td>31.0±1.60*</td>
<td>30.0±1.80*</td>
<td>30.0±1.20*</td>
</tr>
<tr>
<td>T. Protein (mmol/L)</td>
<td>62.0±3.40</td>
<td>49.0±5.0*</td>
<td>50.0±0.8*</td>
<td>50.0±0.8*</td>
<td>52.0±3.8*</td>
</tr>
<tr>
<td>T.Bilirubin (μmol/L)</td>
<td>4.60±1.50</td>
<td>3.80±0.840</td>
<td>4.60±1.10</td>
<td>4.40±1.10</td>
<td>4.20±0.840</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>2.50±0.28</td>
<td>2.20±0.230</td>
<td>2.23±0.10</td>
<td>2.20±0.44</td>
<td>1.50±0.40*</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>5.14±0.16</td>
<td>6.54±1.00</td>
<td>6.36±1.00</td>
<td>5.70±0.54</td>
<td>5.24±1.90</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>37.8±4.50</td>
<td>58.2±3.70*</td>
<td>53.2±6.10*</td>
<td>54.4±3.40*</td>
<td>56.6±3.60*</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>8.28±0.42</td>
<td>6.90±0.99*</td>
<td>6.46±0.62*</td>
<td>6.16±0.43*</td>
<td>6.28±0.94*</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>2.10±0.16</td>
<td>1.30±0.58*</td>
<td>1.04±0.09*</td>
<td>1.18±0.27*</td>
<td>0.88±0.23*</td>
</tr>
</tbody>
</table>

\(b\) = Mean±Standard deviation based on five observations. *P*<0.05 means significantly different from the control.

Figure 1. Stomach of rat treated with aqueous pod extract of *A. nilotica* at 400 mg/kg showing mild congestion (arrow). H and E×200.

necrosis (Figure 3), with mononuclear cell infiltrations. The lesions observed in the kidney were more severe in the groups treated with higher doses of the extract. There was marked interstitial mononuclear cell infiltration and haemorrhages of the lungs in the rats treated with 200 mg/kg dose, while those treated with the highest doses of the extract also had pulmonary oedema (Figure 4) and congestion of the lungs. In the liver there was hepatocellular...
Figure 2. Small intestine of rat treated with aqueous pod extract of *A. nilotica* at 400 mg/kg showing sub mucosal mononuclear cell infiltrations (arrows). H and E x100.

Figure 3. Kidney of rat treated with 200 mg/kg of the aqueous pod extract of *A. nilotica* showing haemorrhage (thick arrows) and Extensive renal tubular degeneration and necrosis (thin arrows). H and E x400.
Figure 4. Lungs of rat treated at 200 mg/kg with aqueous pod extract of *A. nilotica* showing marked interstitial mononuclear infiltration (arrows). H and E ×100.

Figure 5. The liver of rat treated with aqueous pod extract of *A. nilotica* at 200 mg/kg, showing mononuclear cell infiltration (thick arrows) and hepatocellular necrosis (thin arrows) H and E ×400.
necrosis and diffused mononuclear cell infiltrations (Figure 5). The observed lesions appear to be dose dependent. The heart showed intramuscular haemorrhages, mononuclear cell infiltrations, myocardial degeneration and necrosis (Figure 6) in the extract treated rats. The severity of the lesion observed varied with the administered dose. The lesions were very severe in animals treated with the highest dose (400 mg/kg) of the extract.

**DISCUSSION**

Administration of *A. nilotica* pod extract to rats for 21 days resulted in increased AST and ALT values of treated animals. This may be indicative of liver damage (Schalm et al., 1975; Teitz, 1994). The extract also significantly (P<0.05) decreased levels of serum total protein and albumin, which further strengthens the suggestion of liver damage (Jubb et al., 1995). The increase in the value of ALT may also be suggestive of the involvement of other organ and tissues like the heart in the degeneration process (Teitz, 1994). The ALP in this study was not elevated, which may be an indication of non involvement of the bone tissue. The decrease in urea levels and significant increases in creatinine values in this study may suggest that the kidney was adversely affected. Creatinine is produce in the body in proportion to the muscle mass, and its high level indicates renal damage or failure of the kidney to excrete creatinine owing to renal failure (Teitz, 1994). The administration of the extract significantly reduced the cholesterol and triglyceride values of the blood. This could be an indication that the extract affected the cardiovascular
The extract decreased the blood sugar levels significantly (P<0.05). It also induced anorexia in the treated rats. The decrease in blood glucose and triglyceride values following extract administration is an interesting finding which may suggest that this extract could be utilized in treating atherosclerotic and diabetic problems (Taiwo et al., 2005).

The histopathological examination of tissues showed presence of dose dependent severe lesions in the liver, kidney, heart and lungs. The lesion found in the stomach and intestines were milder. The presence of lesions in all these organs and the tissues may be an indication that the active/toxic principle presence in the extract once absorbed was extensively distributed throughout the body (Buxton, 2005). The presence of the lesions in the liver and the kidney may not be unexpected since the liver is the primary organ of biotransformation and the kidney is the main organ of excretion (Baggot, 1977). The extract is known to contain some chemical compounds that are capable of exerting pronounced physiological/pharmacological effects including alkaloids, tannins, saponins and glycosides. It was concluded that the prolonged administration of the aqueous extract of A. nilotica for medicinal purposes should be discourage since it has potential to produce some level of toxicities.

REFERENCES