

Effects of the Aqueous and Ethanolic Extracts of *Cassia italica* Leaf in Normal Rats

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ABSTRACT

The earth is richly endowed with a variety of plants. Most of the naturally occurring plants possess medicinal properties and are thus potential sources of remedies for virtually all human ailments. Although the curative properties of plants are not in doubt, plant medicinal preparations may produce toxic side effects. Unfortunately, a large number of plants known traditionally to possess medicinal properties and which are used as herbal medicines have not been subjected to scientific evaluation. The present study was designed to evaluate the effects of aqueous and ethanol extracts of *Cassia italica* leaf in normal rats. The leaf extracts (aqueous and alcoholic) of *Cassia italica* were administered to rats as a single dose in graded doses ranging from 100mg/kg body weight to 5g/kg body weight orally using intragastric tubes. The control group was given an equal volume of water, and the rats were observed for signs of toxicity and mortality for 72 hours afterward. The acute toxicity studies revealed that dose up to 5g/kg body weight did not show any visible toxic effect. The results of AST, ALT and the haematological parameters studied indicated no threat to health of the rats.

Keywords: *Cassia italica*, leaf extracts, toxicity, rats

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INTRODUCTION

Medicinal plants are widely and successfully used on every continent (Hoareau and DaSilva, 1999). They play a vital role in human health worldwide. Almost 80% of the population relies heavily on traditional medicine (WHO, 1993) In Asia the practice of herbal medicine is extremely well established and documented. As a result, most of the medicinal plants that have international recognition come from this region, particularly from China and India. In Europe and North America, the use of herbal medicine is increasing fast, especially for correcting imbalances caused by modern

diets and lifestyles. Many people now take medicinal plants products on a daily basis, to maintain good health as much as for treatment of illnesses.

In Africa, attitudes towards traditional, herbal medicines vary strongly. One reason for this is the confusion between herbal medicine and witchcraft. The use of medicinal plants is sometimes associated with superstition, and therefore rejected by some people in favour of western medicine. On the other hand, there are millions of Africans who prefer traditional methods of treatment.

The valuable medicinal properties contained in certain plants are not, however, in doubt (Ojo *et al.*, 2006). In recent years, for example, the Chinese plant *Artemisia annua* has become the essential ingredient in a new generation of anti-malaria drugs. The plant is now being grown in East African countries to supply pharmaceutical manufacturers in Europe. The bark of the tree *Prunus africana* is used for traditional treatments of prostate cancer. *Sutherlandia*, a native plant of South Africa, is being increasingly recognised for its herbal usage by HIV/AIDS patients. Other African plants, such as Devil's Claw and African Geranium, are also gaining popularity as herbal medicines, particularly in Europe. Medicinal plants therefore present an important opportunity to rural communities in Africa, as a source of affordable medicine and a source of income.

Plants can, however, have toxic side effects (Pepato *et al.*, 2004). It is necessary to carry out toxicity studies on medicinal plants even though they have been used for decades to determine acceptable from non-acceptable toxicity levels (Jasper *et al.*, 2003). The toxicological evaluation of plants extract generally seeks to determine its possible collateral effects to ensure the safety of use (Idu *et al.*, 2008, Sarkiyayi *et al.*, 2009). Some phytochemicals are able to interfere in the toxicity of herbal plants (Hashemi *et al.*, 2008). And it appears nothing has been published on the toxicity of the leaves of *Cassia italica*. In Adamawa State of Nigeria, the leaves of *C. italica* popularly known as 'ganyen shayi' meaning tea leaf, is used in making tea especially for the people believed to have jaundice or diabetes. Some use it for protection against liver diseases. To this effect, toxicity study of ethanol and aqueous extracts of leaves of *Cassia italica* were conducted in normal rats. Doses of 100-5000mg/kg of crude extracts were employed in a single dose toxicity studies.

MATERIALS AND METHODS

Plant Materials: The mature leaf of *C. italica* were fetched from the vicinity of Federal University of Technology, Yola and Yolde Pate a village in Yola South Local Government area of Adamawa State and botanically identified. A voucher specimen was deposited in the herbarium of the Department of Forestry and wild life management, Federal University of Technology, Yola. The leaves were air dried at room temperature and ground using a laboratory mortar and pestle followed by sieving using a 1mm endocoff sieve. The fine powdered sample was stored in a desiccators at room temperature until required.

Experimental Animals: Male Wistar strain albino rats weighing between 150-180gm needed for this study were purchased from the animal unit of the Nigeria Institute for Trypanosomiasis Research (NITR), Vom. Plateau State, Nigeria. They were fed with standard rat diet and drinking water *ad libitum*.

Chemical and Reagents: All reagents used were of analytical grades.

Preparation of Extract: Portions (100g) of the powdered leaf were extracted with 70% ethanol and water respectively. The mixture was left overnight at room temperature on a shaker. The extract was decanted and the fibrous residue rinsed exhaustively. The extract and the rinsing were pooled together and filtered through whatman No. 1 filter paper and the filtrate freeze dried using a freeze dryer (Adzu *et al.*, 2003). Water was used to reconstitute the solid extract to a desired concentration for the study.

Biochemical Estimation: Diagnostic kits were employed in the analysis of the AST, ALT (Reitman and Frankel, 1957), glucose (Barham and Trinde, (1972), creatinine (Henry, 1974) and urea (Weatherburn, 1967) levels.

Statistical analysis: Numerical data obtained from the study were expressed as the mean value \pm standard error of mean. Differences among means of control and tested groups were determined using Statistical Package for Social Scientist (SPSS 11.0). A probability level of less than 5% ($p \leq 0.05$) was considered significant.

Toxicity Tests: An initial test was done to determine the approximate lethal and non-lethal doses of the extract according to Lorke (1983). Seven groups of three rats each were used in the experiments. The leaf extracts (aqueous and alcoholic) of *Cassia italica* were administered to rats as a single dose in graded doses ranging from 100mg/kg body weight to 5g/kg body weight orally using intragastric tubes. The control group was given an equal volume of water, and the rats were observed for signs of toxicity and mortality for 72 hours afterward (Porchezian and Ansari, 2005). The rats were also observed for other signs of toxicity, such as, excitation and respiratory changes.

RESULTS

The results of treatment with graded doses of aqueous and ethanol extracts of *Cassia italica* leaves on serum levels of AST and ALT of normal rats are shown in figures 1 and 2 respectively. There was no significant difference between the values of ALT in both the extracts. There was slight increase in AST levels that was not significant in rats fed 2000mg/kg body weight when compared to normal rats. However, there was no noticeable increase between the rats fed 2000mg/kg body weight and those rats that were fed 5000mg/kg body weight.

Table 1 and 2 show the results of haematological analysis carried out on normal rats treated with different doses of ethanol and aqueous extracts of *Cassia italica* leaves. At the dose of 600mg/kg body weight of ethanolic extract lymphocyte level dropped significantly ($p \leq 0.05$). Eosinophil increased from 1 to 4%. The rats given up to 5000mg / kg had their hematocrit (PCV), haemoglobin (Hb) and lymphocytes dropped significantly ($p \leq 0.05$), with raised WBC and neutrophil. Similar results were obtained in rats given treatment with aqueous extract of *C. italica* leaf except that

eosinophil is unaffected by the aqueous extract. Also the lymphocytes level was only affected at the dosage of 5000mg/kg body weight.

Acute toxicity study revealed that the ethanol and aqueous extracts had no difference in serum creatinine level up to the dose of 2000mg/kg body weight. There was slight increase at the dose of 2000-5000mg/kg body weight that was significantly ($p \leq 0.05$) higher than the level in untreated rats (Table 3). The urea levels in the normal rats fed 2000 and 5000mg/kg body weight of ethanolic and aqueous extracts showed a significant increased ($p \leq 0.01$) as compared to untreated rats.

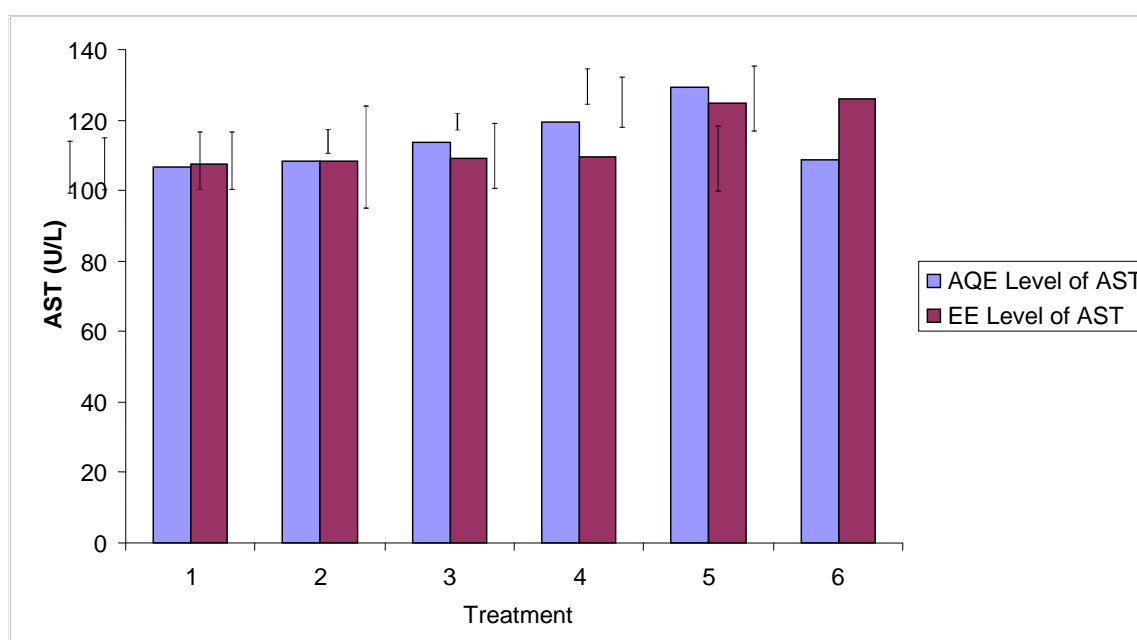


Figure 1: Effect of graded doses of aqueous and ethanolic extracts of *Cassia italica* leaves on AST levels of normal rats.

EE-ethanolic extract, **AQE** - aqueous-extract, **AST** – aspartate aminotransferase.

Values are means \pm SEM: n = 3, P > 0.05 compared to normal.

Treatment 1-----normal
 Treatment 2-----100mg/kg body weight
 Treatment 3-----200mg/kg body weight
 Treatment 4-----600mg/kg body weight
 Treatment 5-----2000mg/kg body weight
 Treatment 6-----5000mg/kg body weight

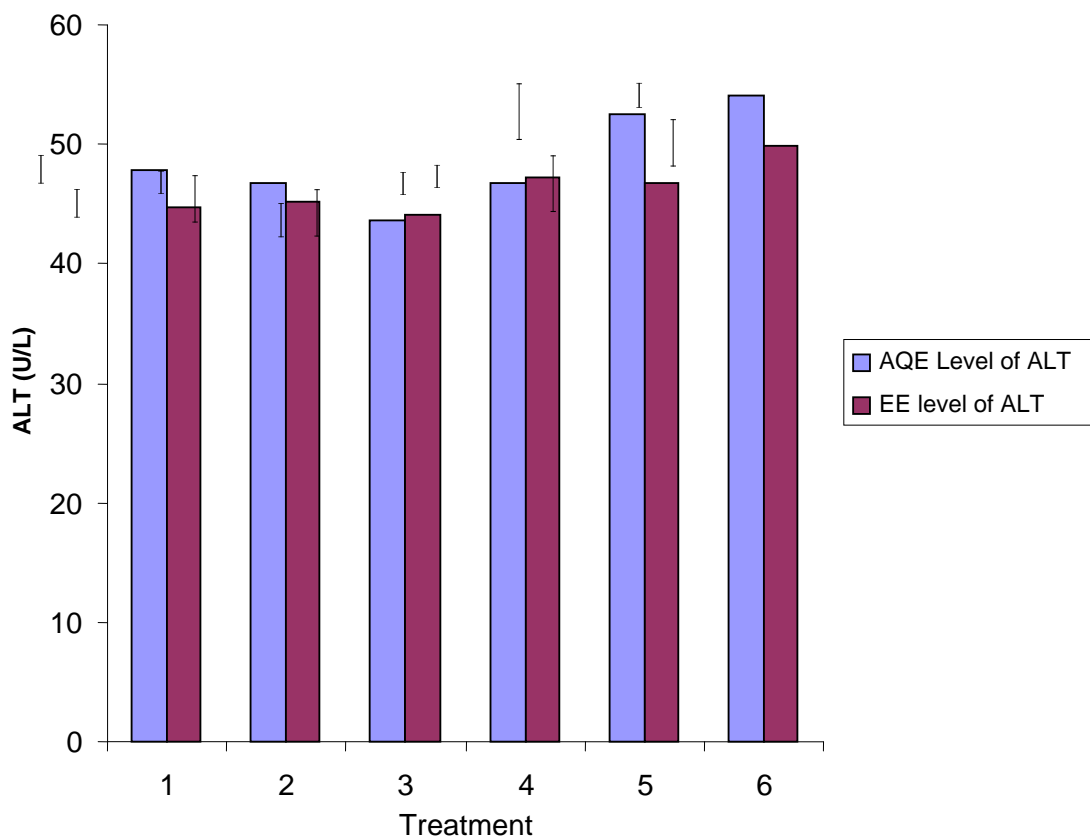


Figure 2: Effect of graded doses of aqueous and ethanolic extracts of *Cassia italica* leaves on ALT levels in normal rats.

Values are means ± SEM: n = 3, *P>0.05 compared to normal.

- Treatment 1-----normal
- Treatment 2-----100mg/kg body weight
- Treatment 3-----200mg/kg body weight
- Treatment 4-----600mg/kg body weight
- Treatment 5-----2000mg/kg body weight
- Treatment 6-----5000mg/kg body weight

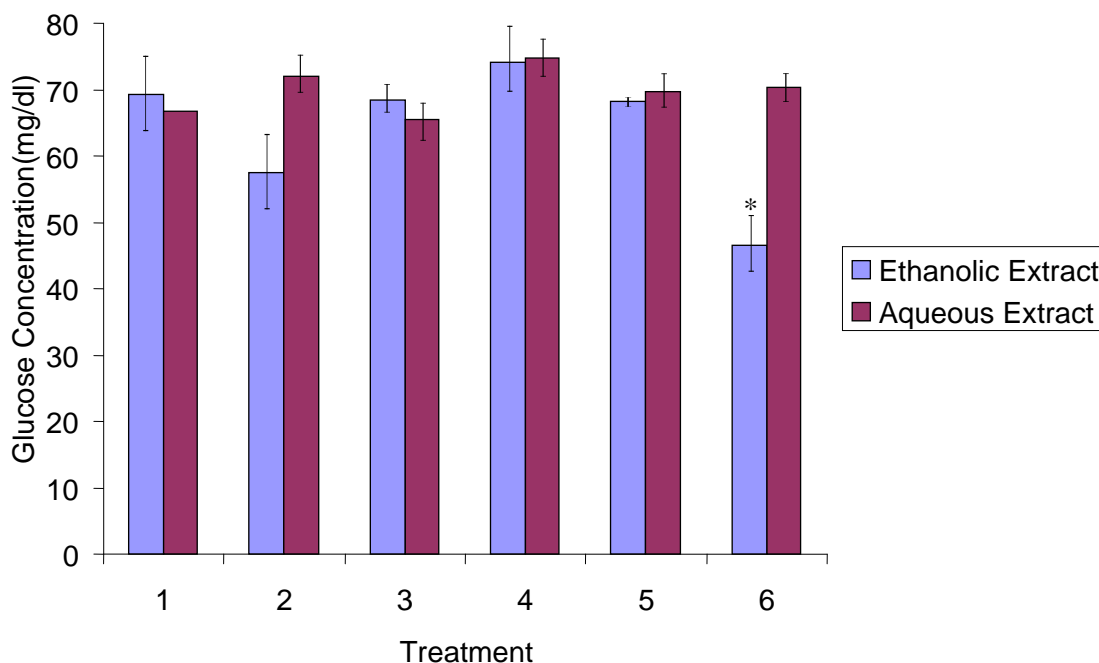


Figure 3: Effect of graded doses of aqueous and ethanolic extracts of *Cassia italica* leaves on glucose levels of normal rats.

Values are means ± SEM: n = 3, *P<0.05 compared to normal.

- Treatment 1-----100mg/kg body weight
- Treatment 2-----200mg/kg body weight
- Treatment 3-----600mg/kg body weight
- Treatment 4-----2000mg/kg body weight
- Treatment 5-----5000mg/kg body weight

Table 1: Dose-dependent effect of ethanol extracts of *C italica* leaves on the haematological parameters in normal rats

| Treatment | PCV (%) | Hb (g/dl) | WBC (m ³) | Lymphocyte (%) | Neutrophil (%) | Eosinophil (%) |
|-----------|-----------|-----------|-----------------------|----------------|----------------|----------------|
| Normal | 48.1± 0.3 | 14.2 ±2.1 | 7.0 ±0.3 | 30.0 ±0.0 | 65.4 ±2.1 | 1.0 ±0.0 |
| 200mg/kg | 47.8 ±1.2 | 14.4 ±0.3 | 6.9 ±0.2 | 30.0 ±0.0 | 64.5 ±3.2 | 1.0 ±0.0 |
| 600mg/kg | 44.2 ±3.6 | 12.5 ±2.3 | 7.8 ±0.3 | 25.5±0.3** | 61.1 ±6.3 | 4.3± 0.2* |
| 2000mg/kg | 41.3 ±2.8 | 11.9 ±1.1 | 7.6 ±0.7 | 20.3±0.7** | 64.7 ±3.8 | 4.5±0.2 * |
| 5000mg/kg | 31.4± .7* | 9.5±1.6** | 10.9±1.1* | 13.3±0.5** | 84.3 ±3.6 * | 1.0 ±0.0 |

Values are means ± SEM; n=3

* Significantly different compared to values obtained for normal rats (p < 0.05)

Table 2: Dose-dependent effect of Aqueous extracts of *C italica* leaves on the haematological parameters in normal rats

| Treatment | PCV (%) | Hb (g/dl) | WBC (m ³) | Lymphocyte (%) | Neutrophil (%) | Eosinophil (%) |
|-----------|------------|-----------|-----------------------|----------------|----------------|----------------|
| Normal | 48.2 ±0.1 | 14.2 ±0.1 | 7.0 ±0.1 | 30.0 ±0.0 | 65.3 ±3.1 | 1.0 ±0.0 |
| 200mg/kg | 47.6 ±0.9 | 13.0 ±0.3 | 7.4 ±0.5 | 30.5±0.5 | 63.4 ±1.7 | 1.0 ±0.0 |
| 600mg/kg | 45.2 ±1.6 | 13.1 ±1.0 | 8.4 ±0.7 | 30.7 ±0.2 | 60.7 ±5.3 | 2.0 ±0.3 |
| 2000mg/kg | 40.5 ±3.1 | 11.7 ±0.4 | 7.4 ±0.0 | 30.3 ±0.1 | 66.1 ±1.9 | 1.0 ±0.0 |
| 5000mg/kg | 31.3±2.3** | 9.1±0.9** | 11.1±0.9* | 14.2±0.7 ** | 85.3±4.7* | 0.0 ±0.0 |

Normal rats were given acute treated with different doses of the extract from *C. italica* leaves and the haematological indices were analysed three days later using EDTA-mixed blood.

Values are means ±SEM; n=3

* Significantly higher compared to values obtained for normal rats (p<0.05)

** Significantly lower compared to values obtained for normal rats (p<0.05)

Table 3: Effect of different concentrations of aqueous and ethanol extracts of *C italica* on serum creatinine and urea levels in normal rats

| Treatment | Creatinine (mg/dl) | | Urea (mg/dl) | |
|-----------|--------------------|--------------|---------------|---------------|
| | EE | AqE | EE | AqE |
| Normal | 0.97 ± 0.10 | 0.97 ± 0.10 | 24.62 ± 1.67 | 24.62 ± 1.67 |
| 200mg/kg | 0.93 ± 0.02 | 1.05 ± 0.02 | 23.65 ± 2.67 | 28.39 ± 1.64 |
| 600mg/kg | 1.14 ± 0.15 | 1.16 ± 0.18 | 27.34 ± 1.19 | 27.92 ± 3.12 |
| 2000mg/kg | 1.54 ± 0.56 * | 1.48 ± 0.56* | 47.24 ± 5.31* | 38.11 ± 5.31* |
| 5000mg/kg | 1.69 ± 0.08* | 1.64 ± 0.08* | 51.65 ± 2.32* | 53.54 ± 2.32* |

EE and AqE (Ethanol Extract and aqueous extract respectively).

Values are means of three determinations ± SEM;

* Significantly higher compared to values obtained for normal rats (p<0.05)

DISCUSSION AND CONCLUSION

The present research has provided first hand information on acute toxicity of *Cassia italica* leaf. The acute toxicity studies revealed that the leaf of *Cassia italica* has LD₅₀ above 5000mg/kg body weight in rats, indicating that the plant is relatively non toxic to experimental animals.

Haematological analysis showed no differences in most parameters examined. Where significant increases were observed the animals had received up to 5000mg/kg body weight. Biochemical analysis also revealed that the study of enzyme activities such as AST and ALT have no significant difference when the rats in treated groups were compared to normal rats. These enzymes were chosen because, in the living systems liver and kidney are considered to be highly sensitive to foreign and toxic agents and AST and ALT are liver marker enzymes (Ju *et al.*, 2008, Hashemi *et al.*, 2008, Nadro *et al.*, 2008; Wilson *et al.*, 2009). The non-protein nitrogen compounds urea and creatinine and end products of protein metabolism must be removed continually to ensure continued protein metabolism in cell (Nwafor *et al.*, 2006). Urea and creatinine, showed slight to significant ($p \leq 0.05$) increases in serum concentration in normal rats fed 1000mg /kg body weight and above, but the values were within physiological acceptable range (Sarkiyayi, 2009).

Normal rats fed with various doses of ethanol and aqueous extract of *Cassia italica* leaf did not produce required hypoglycemic effect (Fig.3). Result showed that only rats fed 5000mg/kg body weight of ethanolic extract of *Cassia italica* leaf exhibited hypoglycaemic ability and at such a high dose, hypoglycaemia could be achieved as a result of poisoning (Marles and Farnsworth, 1994). According to Lorke, 1983 a dose of 5g/kg body weight is of no scientific benefit, so achieving hypoglycaemia with such a dose is of no significance.

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