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Effects of The Aqueous Ethanolic Leaves Extract Of *Celtis Integrifolia* On Liver Function Of Wister Strain Albino Rats

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ABSTRACT

The toxic profile of the aqueous ethanolic leaves extract of *celtis integrifolia* was studied in wister strain albino rats. The rats were administered graded doses (100,150 and 200 mg/kg/day) for 21 days and the effects of the extract on some liver indices were measured. An increase in weight was observed in all treatment groups in the cause of the study.

The acute toxicity (LD_{50}) was estimated to be >1500 mg/kg as no clinical sign of toxicity or death was observed.

At the dose level tested there were no statistically significant increase or decrease in the serum level of alanine aminotransferase, alkaline phosphatise, total protein and albumin. But a high level of aspartate aminotransferase was observed in both the control and the treatment groups.

Key words: *Celtis integrifolia,* toxicity, serum biochemistry.

INTRODUCTION

Natural product has been and has remained the corner stone of health care. Throughout the ages nature has provided humans with essential sources of life, including foods, medicines, shelter and raw materials. Higher plants in particular are good sources of medicine from ancient time and today they continue to play a dominant role in primary health care.

In recent hears the use of herbal remedies has increased because of the belief that they are effective and safe. (Gabriela *el al*, 2009). Within traditional settings reliance on traditional medicinal recourse is dependent on cultural and/or religious preferences.

Plants, vegetables and herbs used as food and in treatment of certain ailment have been accepted currently as one of the main source of drug discovery and development, but only a few of them have been scientifically investigated (especially about their toxicity). (Wanderson, *el al*, 2010). To know the width of margin between

the toxic dose and the therapeutic dose, or what is referred to as therapeutic index.

Therefore, in other to have standard natural plant products, preliminary studies have to be done so as to evaluate possible risk such as undesirable effect (Lienou, *el al*, 2007).

An important medicinal plant species which has been important in the maintenance of man's health is the *Celtis* species, commonly called Hackberry. *Celtis* is a genus of about 60-70 species. They are found all over the world but most predominant in Africa, is the genus *Celtisintegrifolia* (African Hackberry). *C. integrifolia* like other members of the *celtis* specie is found in warm temperate region of the Northern hemisphere. *Celtisintegrifolia* has been reported to contain gamma amino butyric acid (GABA). (Muazu and Kaita, 2008). It contains crude protein and crude fibre. Seventeen amino acids were in varying proportions in the plant. Crude fibre is relatively high in *C. integrifolia*. This implies that in the diet, these vegetable will perform the role of promoting soften stools with increased frequency and regularity of excretion as is characteristics of fibre rich diets.

Ash content represents the index of mineral element present in a sample (Hassan, *et al.*, 2007). *C. integrifolia* has a high ash content of 13.53%. Percentage ash is useful in accessing a plant and gives an idea of the amount of minerals present in a sample. (Micheal and David, 2002). It also contains some mineral element such as iron (Fe), calcium (Ca), sodium (Na), magnesium (Mg), and zinc (Zn), manganese (Mn), copper (Co) and potassium (K). (Kubmarawa *et al*, 2011). The leaves of *C. integrifolia* are boiled to bath children with measles. (Tolu, 2008). The leaves are popularly consumed by certain tribe in Adamawa state. (Kubmarawa, *et al*, 2011).

They are used as ornamental tree valued for their drought tolerance. One modest serving of this vegetable (approximately 50g will be more than enough to satisfy a young adult calcium need). (Sceriano, *et al.*, 1995).

MATERIAL AND METHODS

Plant material

The leaves of the plant, *Celtis integrifolia* (African Hackberry) was collected from Geidam, Yobe state of Nigeria on October 2011 and was authenticated by Professor S.S. Sanusi a botanist at the University of Maiduguri. The aqueous ethanolic (70:30 ethanol:water) leaves extract of *C. integrifolia* was used in this investigation. The fresh leaves of *Celtis integrifolia* were dried under room temperature and processed into fine powder by several rounds of grinding and sieving. The aqueous ethanolic extract was completely dried on a hot plate. The material was stored in a refrigerator at 4^{o}C and protected from light until time of drug administration when appropriate stock solutions were made in distilled water for administration to the experimental animals.

Animals

Wister strain albino rats (180-220) of either sex were used for acute toxicity studies. But only male rats (180-220) were used for the Sub-acute toxicity study. The animals were obtained from

Federal University of technology Yola, Adamawa state, Nigeria. They were fed *ad libitum* with standard feed and had free access to water. They were maintained under standard conditions of humidity, temperature and 12 h light darkness cycle. The animals were acclimatised for a week before the commencement of the studies. All ethical standards for use of these rats have been observed and followed with due permission.

Chemicals

Kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), total protein (TP) and alkaline phosphatase (ALP) used for the biochemical studies were supplied by RANDOX laboratories, United Kingdom.

Acute toxicity studiess

Wister strain rats of different body weight were selected at random and the acute toxicity (LD_{50}) was estimated orally using Lorke's method (1983). Graded doses of plant extract (1000mg/kg and 1500mg/kg) were administered to the animals. The rats were monitored for 72 hours for clinical signs and possible death.

Sub-acute toxicity studies

Twenty (20) male rats were selected at random and divided into four groups of five rats each (n=5/group). The first group is the control group (no extract administered), while the other three groups were administered graded doses of the aqueous ethanolic leaves extract of *Celtis integrifolia*, (100, 150 and 200mg/kg/day) for 21 days.

Preparation of sera samples

On Day 21 of the dosing, all the animals were sacrificed and the blood samples were collected from the animal. The sample were collected in plain plastic tubes and allowed to stand for 3h to ensure complete clotting. The clotted blood samples were centrifuged at 3000rpm for 10mins and clear serum sample were aspirated off and stored frozen.

Serum biochemistry

The following parameters were determined colorimetrically by employing the standard ready-to-use kits of Randox (RANDOX Laboratories Ltd., 55 Diamond Road, Crumlin, Co. Antrim, United Kingdom. BT29 4QY): aspartate amino transferase, alanine amino transferase, alkaline phosphatase, total protein and albumin. The manufacturer's instructions for each biochemical parameter were strictly followed in the course of the investigations.

Statistical Analysis

All data were expressed as Mean±SEM. Results were statistically analyzed by student's 't' test for significant different between group means. 95% level of significance ($p<0.05$), were used for the statistical analysis.

RESULTS

Acute toxicity studies

At the dose level tested, no clinical signs were observed in the treated animal. There was no change in the nature of stool, urine and eye coloration of all treated groups. After 72 hours of treatment, no mortality occurred in the different

group orally treated with aqueous ethanolic leaves extract of *Celtis integrifolia*. The oral LD_{50} value was therefore estimated to be > 1500mg/kg body weight.

Serum Biochemical Observation

The serum level of alkaline phosphatase, albumin, total protein, aspartate aminotransferase and alanine aminotransferase showed no significant change, following treatment with aqueous ethanolic leaves extract of *C. integrifolia*.

However a higher than normal level of aspartate aminotransferase was observed in both the control and treatment groups when (Table 1) compared to the standard reference range (5-40 IU/L)

Table 1. Effects of aqueous ethanolic leaves extract of *C.integrifolia* on some serum liver indices following oral administration for 21 days.

Treatment dose (mg/kg)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	T.P (g/l)	ALB (g/l)
Control	101.2±0.3	78.6 ± 2.6	26.8 ± 0.9	73.4±0.5	33.8±0.8
100	101.0±0.5	80.0±2.8	23.6±0.7	65.6±0.8	31.4±0.5
150	102.0±0.4	76.0±4.9	28.0±1.6	89.8±2.2	33.4±0.9
200	102.0±0.3	79.4±3.2	27.6±0.7	73.4±1.8	31.0±0.5

P is not significant at ($P<0.05$), values are Mean±SEM, (n=5).

ALP- Alkaline phosphatase; AST- Aspartate aminotransferase; ALT- Alanine aminotransferase; T.P- Total protein; ALB- Albumin.

DISCUSSION

This study tested the acute and sub-acute toxicity of the dried leaves extract of *Celtis integrifolia*, which is used in Nigerian traditional medicine and as food by certain tribe. Test doses of *Celtis integrifolia* was orally administered within the range of 1000 to 1500mg/kg in the acute toxicity test. For the sub-acute toxicity test, doses of 100, 150 and 200mg/kg/day were administered to the animals for 21 days.

The result of the acute toxicity study indicated that aqueous ethanolic leaves extract of *C. integrifolia* via oral route with a test dose of up to 1500mg/kg did not produce any sign of toxicity or death in rats, suggesting an LD_{50} above 1500mg/kg by oral route.

In the sub-acute toxicity findings, it was indicated that the aqueous ethanolic leaves extracts of *C. Integrifolia* in doses of 100, 150 and 200mg/kg/day for a period of 21 days did not produce any death or clinical sign of toxicity. But some statistical non-significant changes were

observed in some serum liver indices. In addition, an increased in the body weight of rats was observed during the period of the experiment. This increase in weight can be attributed to access to food and water *ad libitum* and also probably due to the nutritional constituent of the plant.

The levels of alkaline phosphatase, alanine aminotransferase, total protein and albumin showed no significant increase or decrease at $P<0.05$.

A high level of aspartate aminotransferase was observed on both the treatment and control group. This elevation could probably be as a result of leakage aspartate aminotransferase into the blood, but certainly not due to the effect of the treatment plant.

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