Page 447 to 451

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Toxicity Studies of Cassia *Singuena* in Albino Rats

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Abstract

Following aqueous extraction that yielded 12.5% yield, acute and sub-chronic toxicity studies of aqueous leaf extract of Cassia singuena were conducted on Albino rat. The LD_{50} was found to be ≥ 5000mg/kg. There were no significant (P≥0.05) changes between experimental groups A, B and C that received 500, 1000 and 1500mg/kg orally and the control group D that recorded in terms of hematological, serum biochemical, electrolyte and gross pathology changes observed. However there was significant decrease in weight (P< 0.05) in the experimental groups in the 1st week of the 28 days daily administration of the extract, it can therefore be inferred that the extract is safe by OECD standard, having LD_{50} ≥ 5000mg/kg orally in rats.

Key words: *Sub-Chronic Toxicity; Ld50, Hematological; Serum Biochemical; Electrolyte; Cassia singuena*

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Introduction

Cassia simguena is a shrub or a small tree 1-5cm tall, branchless glabrous to densely pubescent crown, open bark reddish, becoming gray- brown and rough with age. Leaves compounds, with 4-10 pairs of oval leaflets, 2.5-cm long, and rachis with a conspicuous gland between each pair of leaflets; rounded at apex. It is also called *Senna Singuena*. It is a species of the drier tropical African region and is often found in thickest, deciduous woodland and savannah (Branan, 1959; Shahina, 1989). It is called "Runhu'' in Hausa.

Haematology is the science of morphology of blood and blood forming tissues, their physiology and pathology (Kelly, 1974). It is natural to expect alterations in blood feature in toxicosis, since blood takes part directly or indirectly in all biochemical processes of the body (Herdistry and weatherall., *et al*. 1974; Sastry, 1994). Usually haemoglobin, packed cell volume, white and red blood cell counts are the parameters measured (Schalm., *et al*. 1975) and the concentration of these parameters can be influenced by ingestion of some toxic plants. (Abatan and Arowolo, 1989).

The aim and objectives of this research was to establish the toxicity profile of *Cassia singuena* by way of Acute toxicity study using the limit does of 5000mg/kg orally in rats, assessment of hematological parameters such as PCV, RBC, WBC, Hb and assessment of liver activities following exposure to different doses of the plant extract.

Materials and Methods

Thirty five (35) Wister albino rats of both sexes weighing between 100 and 200g obtained from faculty of pharmacy, Ahmadu Bello University, Zaria were used for the experiment. They were acclimated for two (2) weeks in Veterinary Pharmacology laboratory Usmanu Danfodiyo University; Sokoto. Three rats out of 35 were used to determine the LD_{50} of the plant using revised up and down procedure at 48hrs interval. The remaining rats were grouped into four (4) with six (6) rats per group and kept in separate cages a week to the commencement of the specific experiment. They fed on pellets of grower's mash (vital feed®) and tap water was provided *ad libitum*.

Plant Collection and Identification

The plant material was collected at Bafarawa town in Isa Local Government Area of Sokoto state with the help of traditional healers. The collected plant material was authenticated to be Cassia singuena by a taxonomist in the botany Unit, faculty of science, Usmanu Danfodiyo University, Sokoto as recommended by Kumar., *et al*. 2001. A simple with voucher No. 20 was deposited in the herbarium of the unit for reference.

Extraction of Plant Material

The air dried plant material was reduced to coarse powder by pounding in a mortar by a wooden pestle. 100g of the powder was weighed using Metler® PK balance and placed in a 2L beaker and 500ml of distilled water was added. The mixture was shaken vigorously for 6hrs at a regular interval of 10mins of shaking and 15mins of rest then the mixture was allowed to stand for 18hrs, shaken again for 10mins and it was then filtered using whatman® filter paper size No. 1 into a clean conical flask.

The filtrate was gradually evaporated at 50°C in an electric drier to dry as was early recommended by Eduardo., *et al*. 2000. The dried extract was then weighed and percentage yield determined as follows:

$$\frac{\text{Weight of the dried filtrate}}{\text{Weight of pulverized material used}} \text{ X } 100 = \% \text{ yield}$$

LD_{50} Determination

Revised Up and Down procedure was used to determine LD_{50} using the Limit Dose test of 5000mg/kg.

Procedure

Revised Up and Down procedure was used to determine the LD_{50}. In this method a Limit Dose of 5000mg/kg is administered orally to a rat. If the rat does not die after 48hours, another one is dose again. If this second one also survives after 48 hours then the third one is again dosed all at 5000mg/kg this third rat survives this dose also for 48 hours then it concluded that the LD_{50} is greater or equal to the Limit Dose of 5000mg/kg used (OECD, 2001).

If the first rat dosed at the limit dose dies before 48hrs then the next rat receive a dose lower than the limit dose. If the second rat dies at this lower dose again then the third rat receives a dose still lower than that of the second rat. But if this third rat survives the dose then the fourth rat receives the dose of the third rat. If this fifth rat that received this dose of the third rat dies then sixth rat now receives lower dose that was given to the second rat. By the time we have three reversals then by the formula provided by the OECD guideline (OECD, 2001) the LD_{50} is calculated, but if the first three rats survive the limit dose then the research needs not to go into Up and Down proper but to conclude that the $LD_{50} \geq$ the limit dose used. (OECD, 2001). In this research three (3) rats were individually dosed with the extract orally at 5000mg/kg at 48hrs interval, after the survival status of the previous one was established along with possible toxicity signs. This was with the aid of sterile 2ml syringes after their weights were determined. The increase or decrease of the dose rate in the up and down proper is by the factor of 3.2.

Sub-acute Toxicity Study

The rats were divided into four groups labeled A, B, C and D with six rats/ group. Groups A-C were administered 500mg/kg, 1000mg and 1500mg/kg of the 30% preparation of the extract of *Cassia singuena* daily by oral route with the aid of 2ml syringes for 28days consecutively, after their weight were determined. Rats in group D served as control and received distilled water of their body weight equivalent volume, considering the distilled water at 5ml/kg by the same route and the same number of days consecutively. Weight taking was weekly.

On the 29th day, the animals were weighed; anesthetized individually using chloroform and two blood samples were taken from each through the hearts. One blood sample in EDTA sample bottle and the other in plain bottle, for haemotological and serum biochemical analysis respectively. The EDTA containing blood samples were analyzed for haematological parameters using auto haemoanalyzer and blood sample in plain sample bottles for serum biochemistry (Kiln, 1980).

Statistical Analysis

The results were presented as mean ± SD and subjected to ANOVA. Difference between means compared were considered significant at $P<0.05$ (Steel and Torie, 1980).

Results

Percentage Yield

The percentage yield of the extract was calculated to be 12.5%.

Acute toxicity study

LD_{50} of this plant using revised Up and Down procedures was found to be ≥5000mg/kg orally in rats.

Sub –acute toxicity studies

Effect of the extract on physical activities.

The rats used in this study showed various dose-dependent signs of toxicity within the first week of the experiment. These toxicity signs observed include depression and inappetance.

Effect of the Extract on Body Weight

Administration of the extract for 4 weeks to rats produced a significant difference statistically ($P < 0.05$), when compared to the control and to themselves, though they slightly lost weight in the first week before they picked up in the second week.

Discussion, Conclusion and Recommendations

Discussion

The low percentage yield of 12. 5% suggests that the plant *Cassia singuen*a contains a lot of fibre that will turn out to be residues after extraction. Acute toxicity study, the LD_{50} of this plant was found to be ≥ 5000mg/kg orally in rats. This is in line with the OECD standard that gives the guarantee level of safety at this level and even higher than that of WHO which recommends the safety level to be 3000mg/kg orally in rats. This plant by both WHO and OECD standards is therefore considered safe for use as a medical plant (WHO, 1991; OECD, 1991).

The manifestation of weight loss and inappetance observed in the rats in the study may be due to the reduced feed and water intakes as a result of exposure to the extract. It could be attributed to the presence of likely anti- nutritional substances such as tannin and saponin that might be present in the extract. However, at day seven, the rats were feeding well and might be due to development of an alternative pathway for the metabolism of the anti-nutritional factor or due to induced production of certain enzymes that can be responsible for the metabolism of the chemical constituents or just adaption. There was no change in hematological parameters in the extract treated groups when compared with the control group ($P>0.05$). This may be an indication that the plant is relatively safe to haemopoetic system at the doses used. (Table 2).

With respect to serum enzymes (Table 3), there were no significant changes (P>0.05) between the experimental and the control groups. This also is in line with the observation that there was no any gross pathological lesion on any of the organs examined. Since increased (ALT) level indicates hepatocellular destruction in dogs, cats and primates as earlier reported by Keneko and Corenelius (1980). And in this study, there was no hepatocellular damage induced by the extract in the experimental rats used. Also, elevations of aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) concentrations in sera of the dogs, cats and primates suggest other specific tissue destruction as earlier reported (Boyd, 1982; Dial, 1995). Since there was no significant elevation in the levels of these enzymes in this study, it can be suggested that there was no significant tissue destruction in the experimental groups.

In this study, there were no significant changes in both cationic and anionic electrolyte concentrations (Table 4), Which may suggest that were was no remarkable effect of the extract on these ions (P>0.05). Sodium and potassium are cations of the extracellular and intracellular fluids respectively and are closely related with water balance and lack of kidney pathology respectively (Coles, 1986; Morag, 1989).

Conclusion

It can be concluded from this study that the aqueous leaf extracts of *Cassia singuena* is safe for use as a medicinal plant by both OECD and WHO standards.

Recommendation

This study was carried out using cold water extraction procedure as practiced by traditional healers. Therefore, it is recommended that further studies should be carried out to investigate hot extraction method and also to find the effect of the extract on histopathology of the tissues of the rats. This plant is traditionally used as an analgesic but this work only established the toxicity profile of the plant. It is therefore also recommend that the analgesic property of this plant should be investigated.

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