African Journal of Biomedical Research, Vol. 7 (2004); 23– 26
ISSN 1119 – 5096 © Ibadan Biomedical Communications Group

Full length Research Article

TOXICITY POTENTIALS OF *CASSIA FISTULA* FRUITS AS LAXATIVE WITH REFERENCE TO SENNA

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The aqueous extract of the pods of Cassia fistula Linn (Leguminosae - Caesalpinoideae), cultivated in Ile-Ife, Nigeria were investigated for pharmacological and toxicological properties. The in-vitro effect of Cassia fistula infusion on isolated guinea-pig ileum was examined. The acute and sub-chronic toxicity of the infusion of C. fistula and Cassia acutifolia Del. Pod-(Senokot tablet) as the reference drug were also determined. The results obtained for C .fistula infusion when compared with senokot tablet showed that the infusion of Cassia fistula pods possessed very low levels of toxicity, having the LD_{50} of 6600mg/kg and also without any pathological effects on the organs examined microscopically. It is therefore concluded from the study that C. fistula pod infusion could be safely utilized as laxative drugs and as a substitute for the official Senna.

***KEY WORDS**: Cassia fistula pods, Senokot tablet, acute and sub-chronic toxicities, gastrointestinal*

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INTRODUCTION:
Cassia fistula Linn (Leguminosae-Caesalpinoideae) is cultivated through-out tropical countries of the world, from West Indies to India, from South Africa to Egypt (Trease and Evans 1985). The plant was even referred to as 'Purging Cassia' in Europe in the thirteenth century (Wallis 1985). *Cassia fistula* has been used extensively in the folklore medicine for the treatment of a variety of diseases. The pulp of the ripe fruits has a mild, pleasant purgative action and is also used as an anti-fungal drug (Kasuko and Nagayo 1951). Butanol extract of the residue of *Cassia fistula* from a 70% alcohol fraction has been shown to have anti-viral effect, while hot water extracts have proved useful in the treatment of uterine, menstrual disorders and fever (Kaji and Khorara 1965; Sen and Shukla 1968). Other ethnomedical uses of *Cassia fistula* plant include its anti-diarrhoeal and anti-dysentery (Manandhar 1989), and in the treatment of diabetes and skin diseases (Chopra *et al* 1992). Recently its hepato - protective activity has been evaluated (Bhakta *et al* 2001). Because of this wide range of therapeutic efficacies of *Cassia fistula* plant, we investigated the effect of the infusion on novelty-induced behavioural activities (rearing -an excitatory locomotory activity, and sedative effect), its *in-vitro*_effect on Guinea-pig ileum and the toxicity potentials on selected organs of rats. This is with a view to examining the possibility of using the pods as a safe substitute for the imported *Cassia acutifolia* Del.

MATERIALS & METHODS: ***Plant Materials**:* The pods of *Cassia fistula* Linn (Leguminosae-Caesalpinoideae) were collected from the plant's natural locations at Ile-Ife in South West Nigeria in the month of May 1998. Identification and authentification were carried out by comparison with established Herbarium specimen with voucher number FH1 187788 in the Forestry Research Institute of Nigeria, Ibadan. The pods were dried, powdered and stored in amber coloured bottles until ready for use.

Preparation of Infusion: 5g of either powdered pods of *C. fistula* or the Reference Senokot (R) tablet was placed in a conical flask unto which 50ml boiling water was added to give 100mg/ml concentration (Elujoba and Iweibo 1988; Ogunti and Elujoba 1993) The infusions were administered to the animals intraperitoneally in the following doses: 800; 1,600; 3,200; 6,400; and 12,800 mg/kg.

Animals: Swiss albino mice (18-20g); Wistar albino rats (180-240g) and Guinea-pigs (210-

250g) were maintained at standard conditions of 12h light/darkness, humidity and temperature in the Department of Pharmacology Animal House, Obafemi Awolowo University, Ile- Ife. They were kept in cages, fed on standard diet with free access to water *ad libitum.*

Drugs: Acetylcholine (BDH), Phentolamine (Sigma) Propanolol (Sigma) Senokot $^{(R)}$ tablet (R eckitt & Colman Products Ltd. U.K)

Acute - Toxicity Determination: For *in-vivo* toxicity test, a pilot study was first conducted to determine the maximum dose of each aqueous infusion that did produce 0% death (800 mg/kg i.p) and the minimum dose that cause 100% death (12,800 mg/kg i.p). Doses were selected within these two doses levels as: 800, 1600, 3200, 6400, 12800 mg/kg for each infusion of *C. fistula* and *C. acutifolia*. Swiss albino mice were fasted overnight, but with free access to water. 5 groups of 4 mice each were randomly selected and the infusions were administered intraperitoneally (i.p.). The signs and symptoms of toxicity referred to as CNS behavioural activities were observed and recorded (Dourish 1982). The number of deaths within 24-48h periods was noted. The LD_{50} value of the infusion was calculated according to the methods of Litchfield and Wilcoxon (1949).

Sub-chronic Toxicity Studies: 28 male Wister albino rats were randomly distributed into seven groups of four. The first group served as the control and received 0.5ml-distilled water. Groups II, III and IV received *Cassia fistula* infusion at doses 250, 500 and 1000 mg/kg i.p. respectively. While groups V, VI and VII received similar doses of Senokot $^{(R)}$ tablet as reference drug. These animals received daily doses for six weeks, sign of toxicity were noted. At the end of six weeks, the animals were sacrificed by decapitation and their liver, kidney, testis and brain were removed and grossly observed. Sections of these organs were also fixed with 10% formalin, embedded in paraffin sectioned at 5u thick and stained with haematoxylin and eosin (Hand E) for histological analysis.

In-vitro Effect of *Cassia fistula* infusion on Guinea-pig ileum: Animals were starved overnight, allowed to have free access to water and then sacrificed. A segment of the terminal ileum (2cm-long) was removed and suspended in 10ml organ bath containing aerated tyrode solution: NaCl 136; KCl 2.7; $MgCl_2$ 1.8; $CaCl_2$1.8; $NaHPO_4$ 0.3; $NaHCO_3$ 12.0 and Glucose 5.6mM which was maintained at 37^{o}C. The tissues were equilibrated for 60min during which the bathing solution was replaced every 10min. At the end of equilibrium, acetylcholine (Ach: $7.3x10^{-10}$ - $1:16x10^{-8}$M) was used to produce dose-response control curve. In the presence of *C. fistula* aqueous infusion (4 and 8mg/ml final bath concentrations) Acetylcholine responses were determined and recorded on Ugo Basile microdynamometer 7050 isotonic transducer. The effect of *C. fistula* infusion in the presence of phentolamine (1×10^{-6}M) and propanolol (2.95×10^{-8}M) was also determined.

RESULTS AND DICUSSION

The infusion of *C. fistula* fruit (4-8 mg/ml) dependently relaxed the ileum. The relaxation was reversible as the issue recovered soon after wash. The senna fruit however did not produce any relaxant effect on guinea-pig ileum. Both phentolamine and propanalol did not block the relaxant property of infusion. This is an indication that the relaxant property exhibited by the infusion of *C. fistula* was not mediated by sympathetic nervous system. Recent studies (Akomolafe 2000) reveals that the relaxant property of both *C. fistula* fruit and senokot tablet infusions on the rat ileum and colon segments were blocked by tolazoline and propanolol indicating that there could be species variation to the action of *C. fistula.*

Agents having anti-diarrhoeal property has been shown to act on the ileum exhibiting relaxant property, while most laxative agents act in the colon through stimulation of the different mesenteric plexuses (Goodman and Gilman 1996). The effect of *C. fistula* and Senokot ® tablet infusion on the ileum of guinea-pig in this study could not support the laxative property probably because the active constituents could not have been released into the lumen of the ileum. The glycoside has to be metabolized (hydrolyzed) in the colon thereby releasing the active anthraquinones component before a laxative effect could be exhibited (Bowman & Rand 1980; Dewick 1997).

In the acute toxicity testing - the LD_{50} values for *C. fistula* pod and senokot tablet were found to be 6,600 mg/kg and 12,000 mg/kg respectively for the intraperitoneal route. The calculation was made according to the method of Litchfield and Wilcoxon (1949). This range is said to be slightly toxic according to Rodricks (1992). The CNS behavioural effect of Cassia fistula on rats showed a calming effect (reduced novelty-induced locomotory activity). All the locomotory, exploratory and stereo-taxic activities exhibited in the control animals were highly suppressed in treated rats (data not shown).

The sub-chronic toxicity studies of the infusion at 250, 500 and 1000 mg/kg oral administration were found not to produce any

change in behaviour, in food consumption and body weight during the six weeks of experiments. Macroscopic inspection of the liver, kidney, testis and brain did not indicate any change in the organs of the treated groups compared to the control rats.

Plate 1: *Photomicrograph of Liver of control rat showing normal structure of hepatic lobule (H & E X148).*

Plate 2: *Photomicrograph of Liver of rat dosed with Cassia fistula at 1000mg/kg (H and E Xl48). The hepatocytes are normal. No pathological lesion is observed.*

Plate.3: *Photomicrograph of Liver of rat dosed with Cassia acutifolia at 1000mg/kg (H & E X148). The biliary cord appears normal. The liver cells show hyperpigmented nuclei as in control group.*

The microscopic or histological examinations of the organs revealed that there was no potential toxicity or damage done to the liver, kidney and the testis. In the liver, there was no observation of necrosis, inflammatory reactions, fibrosis or local fatty degeneration. The biliary cord arrangement still appears not disturbed, which is similar to that of control. (Plates 1, 2, 3).

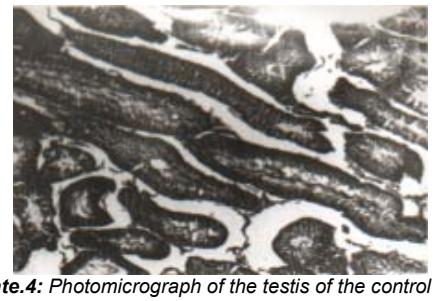

Plate.4: *Photomicrograph of the testis of the control rat (H&E 320X).*

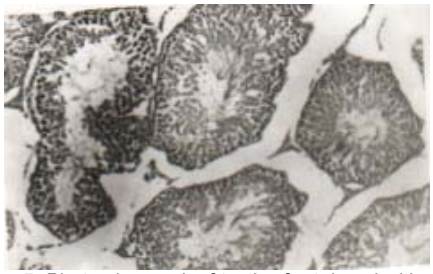

Plate.5: *Photomicrogaph of testis of rat dosed with Cassia fistula at 1 000mg/kg (H&E 320X).*

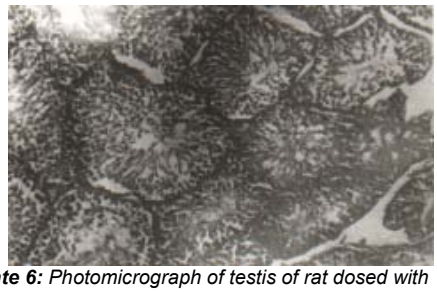

Plate 6: *Photomicrograph of testis of rat dosed with Cassia acutifolia at 1000mg/kg (H & E 320X).*

The testis showed well-preserved seminiferous tubules with evidence of complete spermatogenesis and there was no interstitial fibrosis or inflammation (Plates 4, 5, 6). The kidney section showed preserved renal tubules, the interstitum and the glomeruli. They were devoid of inflammation or fibrosis and the blood vessels were essentially normal (Plates 7, 8, 9).

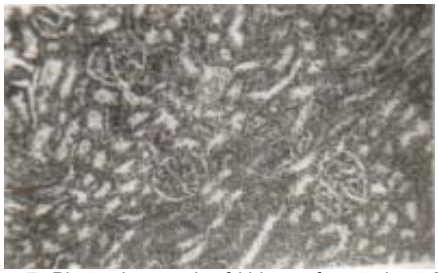

Plate 7: Photomicrograph of kidney of control rat. The section shows normal structure of the glomeruli and proximal tubules (H & E 1 60X).

Plate 8: Photomicrograph of kidney of rat dosed with Cassia fistula at 1000mg/kg. Some of the glomeruli and proximal tubules are widening without injury (H&E 148X).

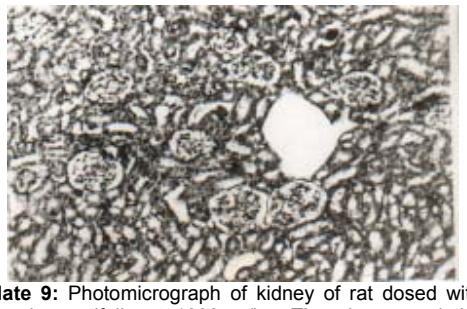

Plate 9: Photomicrograph of kidney of rat dosed with Cassia acutifolia at 1000mg/kg. The glomer and the proximal tubules were widening as in Fig. 8. (H & E 160X).

The laxative properties of both *C. fistula* and *C. acutifolia* tablets have been published (Elujoba *et al* 1999). It was indicated that the pod infusion of the former produced a more significant laxative activity than the latter. The mean % wet feaces (the parameter and index used in comparison) of the former is above 50% level in 12h of oral administration than the latter reference drug. From the above data, as shown in this study and the reports on laxative activity, it could be suggested that *Cassia fistula* pods can be used locally in Nigeria as a substitute for *Cassia acutifolia* pod for laxative activity having shown a considerable bio- activity and a very low toxicity potential in the animals studied.

ACKNOWLEDGEMENT.
Moses. A. Akanmu is grateful to Obafemi Awolowo University for Post-graduate research sponsorship.

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Received: February 2003

Accepted in final form: August 2003