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Some biochemical effects of various doses of aqueous seed extracts of *Cassia occidentalis* in rabbits

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ABSTRACT: This study attempts to investigate the biochemical effects of oral administration of an aqueous *Cassia Occidentalis* seed extract (COSE) in rabbit. Sixteen rabbits weighing between 1.10 – 1.5kg were divided into five groups of four rabbits each. Group 1 served as control, while groups 2, 3 and 4 were given 100, 200 and 300mg/kg body weight of the extract respectively daily for four weeks intragastrically. Blood were collected from the rabbits by marginal ear vein puncture for biochemical analysis. The extract caused an increase in body weight and produced no significant changes in the haematological parameters and plasma cholesterol level. The extract also produced a dose dependent increase in urea level. No histopathological lesion was observed in the liver and kidney.

Key words: *Cassia occidentalis*, Caesalpiniaceae, biochemical, aqueous extract, rabbit.

Introduction

Cassia occidentalis (Caesalpiniceae) is an erect small annual plant growing 4 to 8 metres in height, commonly found in hedges. It is known as Fedegoso in Asian countries (Soukup, 1970), Sanga-Sanga in Hausa; rere in Yoruba and Akede or Agbara in Ibo. Its roots, leaves, flowers and seeds are used in preparation of various compounds like senna syrup, senna tea, senna tincture and the plant has laxative and purgative effects (Blungarten, 1937; Todd, 1967). In Sokoto and other parts of Northern Nigeria, *Cassia occidentalis* leaf extracts has been used in the traditional treatment of febrile illness (Ethkin and Ross, 1983). Previous studies have shown that its leaves exhibit *in vitro* anti-bacterial, anti-malaria and anti-hepatototoxic properties (Perez, 1994; Gasquet, 1993; Saraf, 1994). The seeds are brewed into a coffee-life beverage for asthma and a flower infusion used for bronchitis in the Peruvian Amazon. However, there is death of information on the biochemical effects of *Cassia occidentalis* seed extract (COSE). Thus, this study was designed to investigate COSE extract on some biochemical parameters in rabbits.

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Materials and Methods

Plant Material

Cassia occidentalis seeds were collected within Sokoto metropolis (Rujin Sambo area) during dry season and was authenticated by taxonomist, Mallam Umaru A. of Botany Department, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen was deposited in the herbarium of the Department. The seeds were further air-dried, crushed to powdered form and then kept in airtight container until the time of use.

Preparation of the seed aqueous extract

The aqueous extract of *Cassia occidentalis* seed was obtained using the hot water extraction technique as described by Muyibi et al (2000) in order to stimulate the local procedure. One hundred (100g) of the powdered seeds were weighed into a conical flask and one litre (1000ml) of distilled water was added, the mixture was shaken and then boiled for one hour. It was cooled and shaken before filtration through a sieve and then through Whatman filter paper into a measuring cylinder. The aqueous extract was then concentrated by evaporation using water bath. The residue was stored at 4°C until used.

Animals and experimental treatments

Twenty rabbits weighing between 1.10 – 1.50mg obtained from the animal house unit, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto were used for the experiment. The animals were divided into fouyr groups of five rabbits each and were allowed free access to water and standard diet (Ladokun feeds). Groups 2, 3 and 4 were treated daily with 100, 200 and 300mg/kg of the extract respectively for four weeks orogastrically. Group 1 which served as control received only drinking water equivalent by the same route. The weight of the animal were taken prior to the administration of the extract and subsequently weekly for 4 weeks of the experiment.

Analytical Methods

Collection, preparation and analysis of blood sample

Blood were collected from the rabbits by marginal ear vein puncture on the thirtieth day. A set of blood were collected into heparinised glass capillary tube and used for determination of packed cell volume (PCV), Haemoglobin concentration (Hb), Red Blood Cell (RBC) count and White Blood Cell count (WBC). Another set without anti-coagulant was allowed to clot at room temperature and then centrifuged at 5000g for 30min, and the serum sample used for serum biochemical assays.

Blood analysis

The determination of PCV was by the microhaematocrit method. The haemaglobin (Hb) concentration was determined by cyanomethaemoglobin method (Drabkin and Austin, 1932), White Blood Cell (WBC) and Red Blood Cell (RBC) counts were determined using improved Neubauer Haemocytometer counting chamber (Dacie and Lewis, 1991).

Biochemical assays

Serum glucose levels were determined by glucose oxidase method (Trinder, 1969), total protein by the biuret method (Treitz, 1970), and albumin by the bromocresol green method (Doumas et al, 1982). Total cholesterol levels was estimated by esterase method described by Richmond et al (1973). Serum level of sodium and potassium were determined by modified method of Gbodi (1980). Serum activities of

glutamate oxaloacetate transaminase and glutamate pyruvate transaminase were estimated according to the method of Sigma (1985), alkaline phosphatase by Sigma (1987). Estimation of urea was done by the modified diacetylmonoamine method (marsh, 1965) and creatinine by the Jaffes reaction method (Boid and Sirato, 1948).

Histopathology

Histopathology examination were carried out on the liver and kidney (organs of biotransformation and excretion respectively) from the rabbits. They were fixed in 10% formalin solution. Thin cryostat sections were stained with haematoxylin and eosin, periodic and Schiff reagent with and without diatase respectively. The sections were examined under light microscope at high (X400 objective) power magnification.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) outlined by Steel and Torrie (1980) and significance of difference were assessed at 5% level of probability.

Results and Discussion

The results from this study are shown in Tables 1, 2, 3 and 4. In the present study on this plant, the seed extract administered for four weeks caused significant ($P<0.05$) increase in the body weight of the rabbits receiving the extract (Table 1), this may be an advantage as to the possible use of this plant as an alternative feed in livestock industry. The extract caused no statistically significant ($P<0.05$) differences on the haematological parameters of the rabbits at the tested doses (Table 2). Since the blood is an important index of physiological, pathological and nutritional status, this suggest that the extract probably did not possess anaemic property and also had positive correlation with nutritional quality of the diet and performance of the rabbits. This results is not in agreement with the report of Muyibi et al (2000), that aqueous leaf extract of *Cassia occidentalis* produced significant decrease in the haematological parameter. The differences could be impart, due to the differences in the period of administration amount (concentration) of doses administered and variation in the constituents of leaves and seeds of the plant.

The significant dose dependent reduction in cholesterol (Table 3) might be as a result of the presence of glycosides (Saponins) which form complexes with cholesterols and bile in the gastrointestinal tract leading to reduced blood cholesterol levels. The saponin – like acid complex would disrupt the enterohepatic circulation thereby stimulating the synthesis of bile from cholesterol. This result is in agreement with the report of Milgate and Robert (1995) and Jovanovic et al (1991) that anti-nutritional factors such as saponin and tannins lowers the cholesterol level in the blood.

Table 1: The effect of the aqueous extract of *cassia occidentalis* on body weight of rabbits when compared to pre-treatment value (in grammes).

Treatment	Dose (mg/kg)	Number of Animal	Day 0	Day 8	Day 15	Day 23	Day 30
I	0	5	1134±20	1142±32	1150±25.5	1153±30.4	1166±21
II	100	5	1451±31	1466±23.2	1466±25	1470±32	1471±30
III	200	5	1382±23	1383±23	1388±15	1389±12	1393±15
IV	300	5	1275±32.5	1269±33	1270±8.1	1273±12	1275±12

Table 2: The effects of administration of *Cassia occidentalis* seed extract on haematological parameters in rabbit.

Parameters	**Treatments**			
	1	**2**	**3**	**4**
	Control	**100mg/kg**	**200mg/kg**	**300mg/kg**
PCV (%)	33.5 ± 0.71	34.0 ± 0.13	34.0 ± 1.42	35.0 ± 2.20
Hb (gm/dl)	12.1 ± 0.10	12.2 ± 0.80	11.70 ± 0.70	11.8 ± 1.3
RBC ($X10^{12}$/L)	5.50 ± 0.07	6.70 ± 0.07	6.55 ± 1.10	6.76 ± 1.80
WBC ($X10^{9}$/L	4.80 ± 0.10	4.05 ± 1.5	4.95 ± 1.0	4.35 ± 0.8

Mean ± S.D, n = 5.

Table 3: Effects of graded level of *Cassia occicentalis* on Serum Chemistry of rabbits (Mean ± S.E.M).

Parameters	**Groups/**	**Treatments**		
	1	**2**	**4**	**4**
	Control	**100mg/kg**	**200mg/kg**	**300mg/kg**
Glucose (nmol/L)	9.10 ± 0.1	8.70 ± 0.05	8.87 ± 0.17	8.53 ± 0.12
Total Protein (g/dl)	5.70 ± 0.1	5.85 ± 0.6	5.25 ± 0.1	5.50 ± 0.1
Albumin (g/dl)	3.30 ± 0.1	3.75 ± 0.4	3.30 ± 0.3	3.35 ± 0.2
Globulin (g/dl)	2.40 ± 0.1	2.10 ± 0.2	1.95 ± 1.0	2.15 ± 0.2
Total bilirubin (mg/dl)	0.38 ± 0.08	0.27 ± 0.07	0.33 ± 0.20	0.33 ± 0.07
Conjugated bilirubin (mg/dl)	0.18 ± 1.6	0.15 ± 1.1	0.11 ± 0.05	0.18 ± 0.06
Cholesterol (mg/dl)	$112.6\pm13^{a}.2$	85.20 ± 15.7^{b}	76.5 ± 2.6^{c}	61.15 ± 7.7^{d}
Creatinine (mg/dl)	1.20 ± 0.1	125 ± 0.2	1.05 ± 0.07	1.05 ± 0.07
Urea (nmol/l)	5.05 ± 0.4	6.45 ± 1.1	5.25 ± 0.4	7.60 ± 1.0
K^{+} (nmol/l)	5.9 ± 0.6	5.65 ± 0.8	5.45 ± 0.9	4.5 ± 0.5
Na^{+} (nmol/l)	127.5±20	134.5±5.0	132.0±1.5	125.5±0.7
HCO^{+}_{3} (nmol/l)	19.0 ± 1.4	18.0 ± 1.8	22.0 ± 1.4	23.0 ± 1.4

a,b,c means in the same row with different superscripts differs significantly ($P<0.05$)

The blood glucose levels were not affected thereby suggesting that the extract has no effect on the absorption and metabolism of carbohydrate. However, the increase in the level of urea might be as a result of impairment in the normal kidney function. Kamis et al (2000) reported that malfunction in the filtrations process of the kidney results in the retention of urea and this might explain the increased levels following the administration of the high dose. The extract produced no significant change in the activity of liver

marker enzymes (SGOT, ALP and SGPT) assayed (Table 4). This suggest a non-toxic effect or absence of hepatocellular damage at the investigated concentration.

In conclusion, the study revealed the hypocholesterolaemic effect of the extract especially at high concentration. Work is still in progress to investigate its effect on some other lipids profile as well as its cardiovascular effects.

Table 4: Effects of *Cassia occidentalis* seed extract on serum enzyme activity of rabbit (Mean ± S.E.M).

Parameters	**Treatments**			
	1	**2**	**3**	**4**
	Control	**100mg/kg**	**200mg/kg**	**300mg/kg**
ALP (1.U/L)	17.10 ± 1.83	18.05 ± 0.92	19.35 ± 2.70	
SGPT (1.U/L)	14.5 ± 0.7	15.0 ± 0.5	14.5 ± 0.35	12.0 ± 1.4
SGOT (1.U/L)	17.5 ± 2.1	14.0 ± 0.7	16.0 ± 0.5	15.25 ± 2.2

ALP: Alkaline Phosphase
SGPT: Serum Glutamate Pyruvate Transaminase
SGOT: Serum Glutamate Oxaloacetate Transaminase.

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