



The effects of oral administration of *Croton penduliflorus* seed oil and Depo provera on liver and kidney functions of pregnant Dutch-white rabbits

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ABSTRACT

This study investigated the effects of oral administration of *Croton penduliflorus* seed oil (CSPO) and Depo provera on liver and kidney function of pregnant rabbits. Graded doses of CSPO were suspended in 5% Tween 20 solution. Twenty-five pregnant Dutch-white rabbits at mid-gestation were allocated into 5 groups. Group 1 animals served as control and received 1ml daily of 5% Tween 20 while groups 2-4 received 50, 100 or 150 mg/kg body weight oral dose of CSPO for 10 consecutive days. Animals in group 5 received one intramuscular injection of 2.73 mg/kg of Depo provera. Withdrawal of treatment was followed by 18 hrs fast, and then sacrificed. Blood samples from fasted animals were collected from all groups and analyzed for liver and kidney function parameters. CSPO caused significant ($P < 0.05$) dose-dependent elevation of serum ALT, AST and ALP; dose independent but significant ($P < 0.05$) increases in urea, direct bilirubin and creatinine accompanied by a significant ($P < 0.01$) depression of total bilirubin levels of treated animals. Depo provera resulted in significant ($P < 0.01$) elevation of serum globulin and creatinine levels with significant ($P < 0.05$) depression of total bilirubin. It had non significant effect on the serum electrolytes and marker enzymes. In conclusion CSPO may result in liver damage, biliary obstruction and impaired kidney function.

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Keywords: *Croton penduliflorus*, Depo provera, biliary obstruction.

INTRODUCTION

Croton penduliflorus Hutch (Euphorbiaceae) commonly known as Turk's Cap (Yoruba "Aworoso", Igbo "Ogwuaki" or "Aki Ozara"), thought to originate from Malaysia, is a tropical evergreen plant widely distributed in southern part of Nigeria. It is employed in folklore medicine both as a drastic purgative, as well as a psychotropic medicinal plant (Duke and Ayensu, 1978;

Sofowora, 1982; Olowokudejo et al., 2005). It is credited with antimicrobial, antivenom, antiparalytical, rubefacient and anti-tumor potencies (Odugbemi, 2008). It forms a major component of herbal contraceptives, abortifacient and anti-fibroid concoctions used in the local treatment of fibroids (Adjanohoun et al., 1996; Odesanmi et al., 2006).

Asuzu and Chineme (1987), Asuzu et al. (1988, 1989) indicated that *Croton*

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penduliflorus seed oil had irritant effect on the gastrointestinal tract and induced adverse effects on the internal organs including liver and kidneys and central nervous system of mice. It also induced pharmacological effects on isolated smooth and skeletal muscles and blood pressure. In an earlier study on the plant, oral administration of the ethanolic seeds extract in pregnant and non-pregnant rabbits elicited significant changes in the hematological and metabolic patterns of test animals. Administration to pregnant rabbits caused partial foetal resorption with a dose-dependent severity of effects on internal organs and disruption of normal hormonal pattern (Odesanmi et al., 2006).

Medroxyprogesterone acetate (Depo provera), an orthodox injectable contraceptive, is used in the management of symptomatic uterine fibroids (Venkatachalam et al., 2004). Despite the widespread usage of *Croton penduliflorus* among the local populace as a cheaper, more convenient alternative to orthodox drugs such as RU486 (Abortion pill) and Depo provera in the management of fibroids, information on the metabolic effect remain scanty.

The few reports on the plant seed extracts call to question its effect on the integrity of the metabolic organs of the user, particularly the liver and the kidneys. This study as part of an on-going research on *Croton* seed oil is designed to investigate the effect of oral administration of the Petroleum ether seed extract and Depo provera on some serum marker enzymes, parameters of protein metabolism and electrolytes as indices of liver and kidney function in pregnant Dutch-white rabbits as the animal model.

MATERIALS AND METHODS

Sourcing of materials

Plant material: *Croton penduliflorus* seeds were purchased from the herbal market in Mushin Local Government Area, Lagos,

Nigeria in 2008. The sample was identified and authenticated by Professor Olowokudejo of Botany Dept. of the University of Lagos. A voucher specimen was deposited at the herbarium.

Medroxyprogesterone acetate (Depo provera) 150 mg/ml (NAFDAC Reg. No 04-2310) was obtained from the University Community Pharmacy, CMUL, Idi-Araba, Lagos. All other chemicals were of analytical grade.

Preparation of plant seed extract

Croton penduliflorus seed samples were sorted, cleaned, and the hard outer seed coat removed. The seeds were then oven-dried at 45 °C for 3 days and ground to powder with the Christy-Norris Laboratory Hammer Mill. The powdered seed sample was kept in an airtight container at room temperature until extracted. Five hundred grams of powdered seeds were subjected to soxhlet extraction with 2.5 litres of 40-60 Pet ether unto exhaustion (5 hours). The liquid extract was pooled, concentrated in a rotatory evaporator under reduced pressure and controlled temperature and over-dried at 40 °C. The dried oil was stored in amber bottle and refrigerated until utilized. The percentage yield was calculated thus:

$$\text{Yield} = \frac{\text{Weight of dried oil}}{\text{Weight of powdered sample}} \times 100$$

Weighed portions of the stock seed oil were reconstituted into calculated graded doses of 50, 100 and 150 mg/kg/ml respectively using 5% tween 20 as vehicle.

Experimental animals and study design

Investigation using experimental animals (Dutch-white rabbits) was conducted in accordance with the internationally

accepted principle for laboratory animal use and care (U.S Guidelines – NIH, 1985).

Mature females and males Dutch-white rabbits in the weight range 1-1.6 kg were obtained from the laboratory animal centre of the College of Medicine, University of Lagos, Idi-Araba and Nigerian Institute of Medical Research Yaba. The animals were housed in metabolic cages with males separated from females in the metabolic laboratory under ambient temperature and 12 hrs light and dark periodicity. They were fed commercial rabbit pellets (Niemeth livestock Feeds, Ltd. Ikeja) and water *ad-libitum* and allowed to acclimatize for 4 weeks. The females were observed for the next 7 days for signs of “heat” as marked by restlessness and swollen vulva. Labeled rabbits ‘on heat’ were introduced into the male cages for mating and then returned to their former cages once mating was confirmed. The date of mating was recorded. Successful ‘service’ and pregnancy were confirmed by easy loss of hair with a gentle pull and the feel of marble – shaped lumps under the belly 14 days after mating (mid-gestation).

Twenty five rabbits out of the pregnant female weighing, 1-1.6 kg were selected and randomly allocated to 5 Groups of 5 rabbits/group such that the difference in average weight did not exceed 5 g.

Rabbits in Group 1 served as control and were each given daily oral dose of 5% tween 20 (vehicle) only via catheter. Groups 2-4 received doses of 50, 100 and 150 mg/kg body weight of *Croton penduliflorus* seed oil (CPSO) respectively for 10 consecutive days from Day 15 of pregnancy (mid gestation). Rabbits in group 5 were each given intramuscular injection of the pharmacological dose (2.73 mg/ml) of Depo provera once at mid-gestation. All animals were weighed twice weekly and allowed feed and water *ad libitum* throughout period of assay.

Treatment was terminated on Day 24 of gestation. All groups were fasted overnight (18 hrs) then sacrificed after anesthesia with diethyl ether. Blood samples were collected from each animal via cardiac puncture into sterile non-heparinised bottles and allowed to clot at room temperature. The clot was removed and the fluid centrifuged at 2,500 g for 10 minutes to obtain the fasting serum. The serum for each group was pooled and stored in the biofreezer until analyzed. The serum samples from each group were subjected to biochemical analysis. Fasting serum levels of L-alanine aminotransferase, L-aspartate aminotransferase, alkaline phosphatase, albumin, globulins, and urea were determined by standard protocols using the screen master automated spectrophotometer and corresponding reagent kits (Tietz, 1990; Randox, 1993; Yakubu et al., 2006). Creatinine and bilirubin were determined using the modified method of Henry (1974). Sodium and potassium ion concentrations were determined by flame photometry (Yakubu et al., 2006) while bicarbonate ion concentration was determined by titrimetry method (Oze et al., 2007)

Statistical analysis

All results were analyzed using students t-test and ANOVA with the aid of SPSS (ver. 15) software package. The level of statistical significance was taken as $P < 0.05$.

RESULTS

The percentage yield of *Croton penduliflorus* seed oil (CSPO) was 10.5%.

Tables 1-3 show the effects of oral administration of *Croton penduliflorus* seed oil (CPSO) and medroxyprogesterone acetate (Depo provera), on indices of liver and kidney functions in treated pregnant Dutch-white rabbits.

Croton penduliflorus seed oil (CPSO) elicited highly significant ($P < 0.01$) dose-

dependent elevations of serum L-alanine and L-aspartate aminotransferases and alkaline phosphatase enzymes of treated animals compared to both Depo provera and control groups. Depo provera caused no significant alterations in the serum-levels of the marker enzymes (Table 1).

Effect on protein metabolites

CPSO caused non-dose-dependent, but significant increases in blood serum levels of urea, direct bilirubin and creatinine accompanied by a highly significant ($P < 0.01$) depression of total bilirubin levels of treated rabbits compared to control (Table 2). An initial non-dose-dependent depression of fasting globulin levels from the 50-100 mg/kg dose levels was replaced by the elevation at 150 mg/kg body weight dose with a shift in the protein pattern resulting to further increases in serum albumin levels of treated rabbits (Table 2). Depo provera exhibited a

similar pattern to the 50 mg/kg dose of CPSO causing a highly significant ($P < 0.01$) depression of serum levels of globulins and total bilirubin coupled with a significant elevation of creatinine.

Effects on serum electrolytes

CPSO at 50 mg/kg caused significant elevation of serum sodium, potassium and bicarbonate ion concentrations of test groups, but the effects were less marked at higher doses compared to control animals while Depo provera has a non-significant effect on the electrolyte levels of treated animals (Table 3).

DISCUSSION

Increases in blood serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) could be the result of proliferation, an increase in turnover or

Table 1 : Effect of oral administration of varied doses of *Croton penduliflorus* seed oil and Depo provera on fasting serum concentrations of L-alanine aminotransferase, L-aspartate aminotransferase and alkaline phosphatase of pregnant Dutch-white rabbits¹.

Group/ Treatment	Dose (mg/kg B.wt)	L-Alanine aminotransferase (IU)	L-Aspartate aminotransferase (IU)	Alkaline phosphatase (IU)
Control	Nil	7.00 ± 0.20	13.00 ± 2.00	9.00 ± 2.00
CPSO (I)	50.0	13.67 ± 9.50*	20.00 ± 8.88*	33.67 ± 4.50*
(II)	100.0	18.33 ± 7.57*	25.00 ± 2.00*	58.67 ± 4.50*
(III)	150.0	41.00 ± 9.00**	90.67 ± 3.22**	63.67 ± 3.00**
Depo provera	2.73	8.30 ± 0.20	12.33 ± 1.53	10.00 ± 1.00

¹ Values represent Mean ± SD for 5 rabbits and triplicate determinations

* $P < 0.05$ ** $P < 0.01$ significant difference compared to control

CPSO: *Croton penduliflorus* seed oil.

Table 2: The effect of oral administration of varied doses of *Croton penduliflorus* seed oil on fasting serum level of albumin, globulin, urea, creatinine, total and direct bilirubin in pregnant Dutch-white rabbits¹.

Group/ Treatment	Dose (mg/kg)	Albumin (g/l)	Globulin (g/l)	Urea (mg/l)	T. Bilirubin (μ mol/l)	D. Bilirubin (μ mol/l)	Creatinine (μ mol/l)
Control	Nil	35 \pm 5.0	46.7 \pm 0.58	14.5 \pm 0.4	97.47 \pm 12.6	8.83 \pm 0.75	72.7 \pm 0.7
CPSO (i)	50.0	33 \pm 1.0	12.67 \pm 3.1*	23.17 \pm 0.55*	30.57 \pm 5.5**	12.03 \pm 6.6	165.0 \pm 23.1*
(ii)	100.0	37.0 \pm 2.0	36.00 \pm 5.0*	16.97 \pm 0.12*	38.97 \pm 5.05*	14.43 \pm 3.6	148.8 \pm 28.5*
(iii)	150.0	41.0 \pm 1.0	76.7 \pm 2.01**	25.7 \pm 1.02*	30.22 \pm 16.6*	11.47 \pm 2.15	165.00 \pm 4.00*
Depo provera	2.73	36.0 \pm 2.0	25.67 \pm 3.51**	15.17 \pm 0.8	36.57 \pm 1.45*	5.03 \pm 0.85*	346.46 \pm 0.6**

¹ Values represent Mean \pm SD for 5 rabbits and triplicate determinations

*P<0.05, ** P<0.01 significant difference compared to control

CPSO: *Croton penduliflorus* seed oil

damage or in the case of enzyme synthesis (induction) or to reduce clearance from the plasma (Mayne, 1994; Yakubu et al., 2005). ALT and AST are sensitive indicators of damage to cytoplasm or mitochondrial membrane (Yakubu et al., 2005). The concurrent dose-dependent increases of ALT, AST and alkaline phosphatase (ALP) by the pet-ether seed extract of *Croton penduliflorus* (CPSO) suggest liver cell damage which is characterized by release of enzymes, particularly transaminases from damaged hepatocytes resulting in elevation of serum levels of the marker enzymes (Olaleye, 2006).

Secondly, the non-dose dependent but significant increases in the serum levels of urea, direct bilirubin and creatinine are indications of impaired hepatic secretory function which may be accompanied by a high alkaline phosphatase activity. Increased plasma activity has also been associated with cholestatic liver disease where ALP synthesis is increase and the enzyme within the biliary tract is regurgitated into plasma due to biliary obstruction (Yakubu et al., 2006). Increased plasma urea and creatinine may be due to factors which include a high protein diet, absorption of amino acids and peptides from

digested blood after haemorrhage into the gastrointestinal lumen or soft tissues or increased catabolism, due to starvation, sepsis tissue damage or steroids treatment. Tissue creatine is largely derived from endogenous source by tissue creatine breakdown and is also related to tissue mass. Significant elevations of either urea or creatinine indicate impaired glomerular and impaired kidney function (Crook, 2006a). The highly significant elevation of both urea and creatinine levels of rabbits treated with *Croton penduliflorus* seeds oil suggest impaired kidney function due to reduced glomerular filtration. Some drugs and other toxins are hepatotoxic sometimes directly and sometimes due to hypersensitivity reaction in which case, the damage is not dose-related (Crook, 2006b). The dose independent increases in some metabolic parameter of CPSO-treated animals suggest that the plant extract might have elicited hypersensitivity reactions in the treated rabbits. Physical observations during the present study of symptoms of irritation by the plant extract on the skin and nasal cavities of these exposed animals support the inference. The assertion

Table 3: Effect of oral administration of varied doses of *Croton penduliflorus* seed oil and Depo provera on serum concentrations of sodium, potassium, and bicarbonate ions of pregnant Dutch-white rabbits¹.

Group/ Treatment	Dose (mg/kg B. wt)	Serum electrolytes		
		Sodium ion mmol/l	Potassium ion mmol/l	Bicarbonate ion mmol/l
Control	Nil	120.0±1.0	5.60 ± 0.2	16.0±0.5
CPSO (I)	50.0	152.00±2.0**	11.73±0.15**	18.0±0.25
(II)	100.0	122.33 ± 9.7	10.27±1.75*	16.3±0.58
(III)	150.0	124.00±4.00*	7.4±0.6*	18.33±0.68
Depo provera	2.73	127.0±2.00*	6.1±0.1*	15.00±1.00

¹ Values represent Mean±SD for 5 rabbits and triplicate determinations

* P<0.05 ** P<0.01 significant difference compared to control

Legend CPSO- *Croton penduliflorus* Seed oil

is further corroborated by an earlier report by Asuzu and Chineme (1987) on the gastrointestinal irritants effect of the seed oil in mice. The irritant effects have also been associated with the presence of volatile oils, carbon disulphide, ether and other unidentified secondary metabolites. Phytochemicals so far isolated from croton species include diterpenes corresponding to clerodanes, cembranoids, labdanes, trachylobanes and alkaloids. Cytotoxic effects have also been observed in assays with alkaloids (taspine) and phorbol esters, Elevation of electrolyte levels particularly sodium ions at 50 mg/kg/dose which was not sustained at the higher doses is another indication of hypersensitive reaction as well as disturbed homeostasis.

The lower values at higher doses (> 100 mg/kg) may be as a result of amelioration of the effect by other components of the extract whose effects become more prominent at the higher doses and/or a form of adaptation. It also has been reported that even though phorbol esters, one of the recognized

components of the plant extract may exert potent co-carcinogenic activity, still among them, many may exert beneficial biological effects (Antonio et al., 2007). Flavonoids and antioxidants have also been reported for other parts of the plant and may contribute to the ameliorating its effects (Antonio et al., 2007).

Conclusion

Oral administration of the petroleum ether seed extract of *Croton penduliflorus* may result liver cell damage, biliary obstruction and impaired kidney function in users.

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