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THE COMPARATIVE EFFECTS OF AQUEOUS EXTRACT OF *TETRACARPIDIUM CONOPHORUM* SEEDS AND PROVIRON ON THE BIOCHEMICAL PARAMETERS OF MALE GUINEA PIGS

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Abstract

The phytochemical screening and the effects of the aqueous extracts of the seeds of *T. conophorum* on the biochemical parameters of male Guinea pigs were investigated. The Biochemical parameters were assayed using Randox Diagnostic kits, Phenophthalin and colorimetric methods. The phytochemical screening was carried out using standard procedures.
Phytochemical investigations revealed the presence of flavonoids, tannins, carbohydrate, alkaloids, terpenoids, steroids, volatile oils, saponins and cardiac glycosides. The aqueous extract of *T .conophorum* seeds (100-400mg/kg) caused a statistically significant ($P< 0.05$ ANOVA) decrease in the levels of total cholesterol, creatinine, urea, uric acid, prostatic, alkaline, and acid phosphatases. The highest reduction effect was obtained with uric acid at 400mg/kg of *T. conophorum* extract while the least effect was observed in total cholesterol. These effects were dose- and time-dependent. This shows that the seeds of *T .conophorum* have hepatoprotective, nephroprotective and cardio protective properties. The study therefore, supports the claim on the use of the seeds of this plant by traditional medicine practitioners as a Cardio protective agent. Although further studies need to be done to isolate, identify and characterize the active principles in the seeds extracts of this plant.

Key words: *T. conophorum*: proviron: biochemical parameters: male guinea pigs.

INTRODUCTION

Tetracarpidium conophorum Mull (Arg), (Euphorbiaceae) is commonly known as 'African Walnut' or 'Conophor'. It is a west Equatorial perennial climber often found in the most forest zones of sub- Saharan Africa. It is widely distributed in the southern part of Nigeria, (Dalziel, 1937). *T. conophorum* is known as 'Ukpa' (Igbo) and 'awusa' or 'asala' (Yoruba); in the Littoral and western Cameroon, 'kaso' or 'ngak', (Ajaiyeoba and Fadare, 2006).

In southern Nigerian traditional medicine, *T. conophorum* is used as male fertility agent, to improve fertility and increase libido. The oil from the nut has been found useful in the formulation of wood vanish, stand oil, vulcanized oil for rubber and leather substitute. The stem, root, and leaves have been found to exert antimicrobial activities (Ajaiyeoba and Fadare, 2006). The seeds of *T. conophorum* is useful in the production of snacks and delicacy, (Oke and Fafuns, 1975; Adebona *et al.*, 1988; Akpuaka and Nwankwo, 2000). Two isolectins,

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Agglutin 1 and 11 were characterized from the seeds extract (Animasaun *et al.*, 1994). The presence of oxalate, phytates, tannins, proteins, fibre, oil and carbohydrate in the seeds has been reported (Enujugha, 2003; Enujiugha and Ayodel-Oni, 2003).

Although the *T .conophorum* is used by traditional medicine practitioners in Nigeria in treatment of many diseases, there is no scientific report on its effects on the biochemical parameters in animal models. In our previous study, on the effects of *T .conophorum* seeds extract on the hormonal and sperm parameters of male guinea pigs, it was found out that *T .conophorum* seed showed increases in testosterone and sperm count of male guinea pigs, which were very significant and comparable to the observed effects with proviron (Obianime and Uche, 2009; Obianime and Uche, 2010). It is in the light of this, that this study sought to establish for the first time, scientific information on the comparative effect of Proviron and *T .conophorum* on biochemical parameters of male guinea pigs.

MATERIALS AND METHOD

Adult male guinea pigs of average weight 300-600 g were obtained from the animal house of University of Port Harcourt, housed in a cage of five animals per cage and allowed to acclimatize with the new environment for 10 days. The animals were properly fed on elephant grass (*Pennisetum purpureum*) throughout the experimental period.

All the chemicals used were of analytical grade.

The seeds of *Tetracarpidium conophorum* were collected in June 2008 from the southern part (Osun State) of Nigeria. The plant was authenticated by Edwin Wosu at Botany Herbarium of University of Port Harcourt Nigeria, where voucher specimen was maintained.

The plant seeds collected were boiled at 100 ^{0}C 1h. The seeds were dried in the oven at 29^{0}C for 2 h. The dried seeds were ground with hammer mill and the fine powder macerated in aqueous ethanol (20:80) at room temperature for 72 h. The yields of the extract were obtained by evaporating to dryness using rotary evaporator. The extract (210 g) was stored in the refrigerator at 4 ^{0}C for subsequent reconstitution and use.

Chemical tests were carried out on the extracts using standard procedures to identify the constituents (Trease and Evans, 1989; Harborne, 1978) by characteristic colour changes as described (Sofowora, 1998; Odebedy and Sofowora, 1978). Briefly, formation of brownish green coloration on addition of 3 drops of ferric chloride to sample confirmed the presence of tannins; Formation of yellow coloration which disappears on standing when 5 ml of dilute ammonia solution and concentrated sulphuric acid were added sequentially to portion of the extract confirms the presence of flavonoids; presence of steroids was confirmed by colour change from violet to blue on addition of 2 ml acetic anhydride and 2 ml sulphuric acid to 0.5 g plant extract; Terpenoids were confirmed by formation of reddish brown coloration of the interface on addition of 2 ml chloroform and concentrated sulphuric acid. Saponins were confirmed in the plant by the frothing and haemolysis tests and cardiac glycosides were confirmed by formation of brown ring of interface on addition of 2 ml glacial acetic acid containing 1 drop of ferric chloride solution and 1 ml concentrated sulphuric acid; presence of alkaloids were confirmed by Dragendorff's reagent which formed a reddish brown precipitate with the sample.

Biochemical assay

Evaluation of biochemical parameters

The animals were divided into ten groups of five animals each. Group 1 - 7 were used for dose- dependent studies. The animals in group 1 - 7 received different doses of the extract (100 – 400 mg/kg/day) for 96 hours after which they were sacrificed (table 1 b). Groups 8 - 15 were used for time –dependent studies for a period of 28 days. Group 8 - 11 animals received a fixed dose of the extract (100 mg/kg/day) over a period of 7, 14, 21, 28 days respectively (table 2 b). While group 12 – 15 animals received a fixed dose of proviron (25 mg/kg/ day) over a period of 7, 14, 21 and 28 days respectively (table 2 b). Group 16 animals were used as control (table 2 b). At the end of each treatment period, the animals from different groups were anesthetized with diethyl ether. The blood samples were collected by cardiac puncture, for biochemical parameters - phosphatases, urea, creatinine, total protein, total cholesterol and uric acid. These were assayed using Randox Diagnostic kits, Phenolphthalein and colorimetric methods. Sample serum was separated from the cells, centrifuged at 3400 r for 10 minute and used for the assays.

Statistical analysis

Data were expressed as Mean ± Standard Error of Mean (SEM) of five observations. Statistical analysis of data was performed using analysis of variance (ANOVA), the differences between mean accepted as significant at $P < 0.05$ (ANOVA).

RESULTS AND DISCUSSION

Phytochemical screening revealed the presence of steroids, flavonoids, alkaloids, steroids, cardiac glycosides, volatile oils, terpenoids, tannins and saponins.

This study showed that the aqueous extract of the seeds of *T. conophorum* caused dose- and time- dependent decreases in the biochemical parameters such as: ACP, ALT, Urea, Uric acid, Creatinine, Total cholesterol, Protein, Alkaline, Acid, and Prostatic Phosphatases (Tables 1 and 2).These effects were statistically significant at $P < 0.05$ (ANOVA).Treatment with cadmium caused increase in the levels of acid phosphatase, prostatic acid, Uric acid and creatinine (figures 1 and 2). This effect was inhibited by pre- treatment with *T. conophorum* (figures 1 and 2). However, there was no significant change in the levels of total protein, alkaline phosphatase and total cholesterol on treatment with cadmium (Figures 1and 2;
Tables 1and 2).

The aqueous extract of 100 mg/kg of *T. conophorum* seed caused significant decreases in the levels of ACP, PA, ALP, UA, TP, urea, creatinine and total cholesterol in male guinea pigs (tables 1 and 1b). There were increases in the levels of ACP, PA, ALP, UA, TP, creatinine, and total cholesterol when 1 mg/kg of cadmium was administered to the animals (group 6; tables 1 and 1b). However, this increase in the levels of biochemical parameters was inhibited by pre-treatment with 200 mg /kg *T. conophorum* (group 7; Tables 1 and 1b).

By reducing the total cholesterol levels, it indicates that this plant can exert anti- lipidemic properties. By lowering the levels of phosphatases and creatinine, it indicates that *T. conophorum* has hepatoprotective and

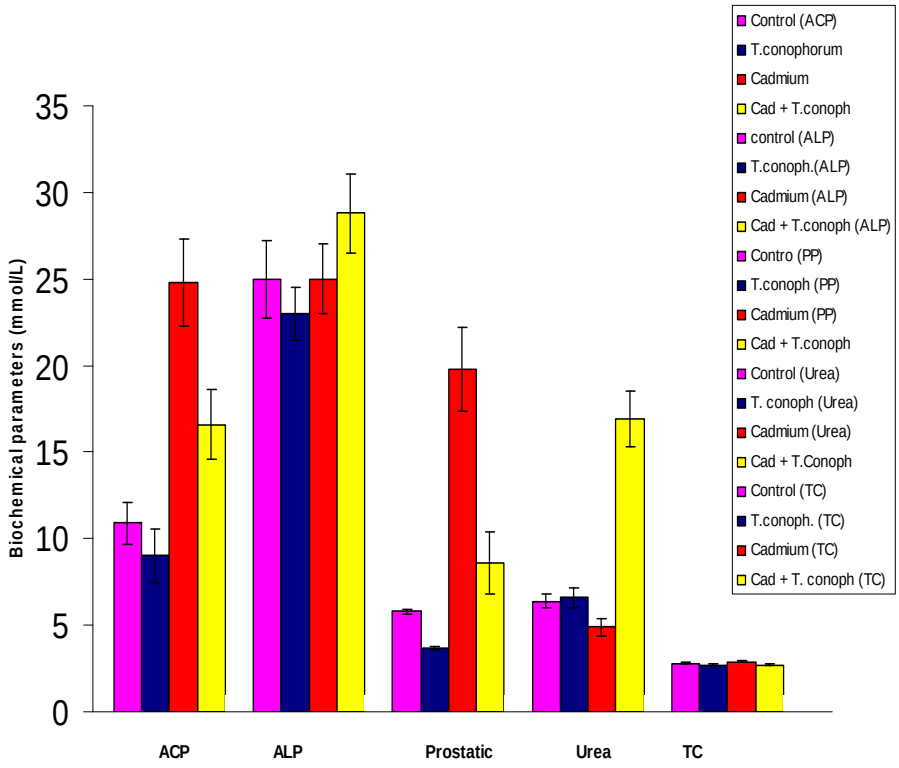

Fig 1: The effect of 100mg/kg of T.conophorum on the biochemical parameters of male guinea pigs

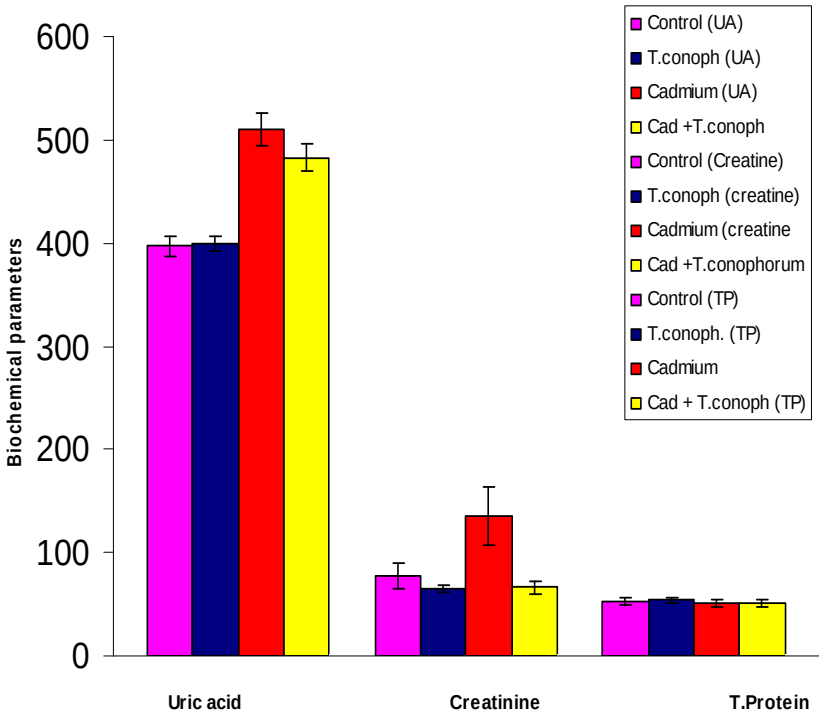

Fig 2: The effects of 100mg/kg of *T.conophorum* on the biochemical parameters of male guinea pigs

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Table 1: The dose-dependent effects of *T.conophorum* on the biochemical parameters of male guinea pigs.

DOSE (Mg/kg)	ACP	PA	ALP	UA	TP	UREA	CREATININE	TCH
Control	10.9± 1.2	5.8± .15	25.00± 2.25	396.8± 10.5	52.5±3.70	6.38± 0.4	77.3± 12.9	2.8 ±.03
100	10.2± 0.7	[a] 3.9± .09	27.0±3.0	[a] 326.5±6.2	52.3±2.5	5.9± 0.3	[b] 5.0± 9.0	2.7±0.05
200	[a] 9.0± 1.6	[a] 3.7± .08	[a] 23.0±1.52	[a] 399.8±7.0	53.7± 3.0	6.6±0.60	64.3± 3.50	2.7±0.05
300	10.8± 1.9	[a] 4.3± .10	25.7± 1.50	[a] 392.3± 11.4	53.7±2.30	6.7±0.60	[a] 83.7± 13.0	2.7±0.05
400	11.1± 1.0	5.8±0.62	[a] 21.5± 1.30	[a] 384.0±44.0	[a] 57.0±3.10	6.1±0.50	[a] 66.5± 4.50	2.7±0.05
Cd	[a] 24.8±2.50	[a] 19.8±2.40	25.5±2.00	[a] 510.5±15.0	51.8±4.0	4.9±0.5	[a] 136.0±28.0	2.9±0.06
Cd+Tc	16.6± 2.0	[a] 8.6±1.80	28.5±2.30	[a] 482.5±13.0	51.2±4.0	16.9±1.60	[a] 66.3±6.00	2.7±0.05

Results expressed as Mean values and SEM; n = 5; [a] P< 0.05; [b] P <0.001 (ANOVA).
Cd represents cadmium; Tc, *Tetracarpidium conophorum;* 0.00 control; ACP, PA, ALP, prostatic and alkaline phosphatase respectively; UA uric acid; TP total protein; TCH total cholesterol.

Table 1b: Doses of the administered extracts

Group	**Dose (mg/kg)** *T.conophorum*
Group 1 (control)	0.5 ml/kg Normal saline
Group 2	100 *T.conophorum* Tc)
Group 3	200 *T.conophorum*
Group 4	300 *T.conophorum*
Group 5	400 *T.conophorum*
Group 6	1 mg/kg Cadmium (Cd)
Group 7	1 mg/kgCd + 200mg/kg (Tc)

Table 2: The Time-dependent effects of *T.conophorum* on the biochemical parameters of male guinea pigs.

TIME (days)		ACP	PA	ALP	UA	TP	UREA	CREATININE	TCH
0.00		10.9±1.2	5.8±0.2	25.0±2.3	396.8±10.5	52.5±3.7	6.4±0.4	77.3±12.9	2.8±0.03
7:	T.c	10.2±0.7	[a] 3.9±0.1	25.0±1.7	[a] 326.7±10.5	52.3±2.5	5.9±0.3	[b] 55.0±9.0	2.7±0.03
	PV	14.3±1.5	9.8± 1.3	23.0±1.5	[a] 414.0±6.2	55.7± 2.3	7.2±0.5	[a] 59.7± 10.1	2.7±0.03
14:	T.c	9.9±1.8	4.3±0.2	22.0±1.3	391.3±11.6	54.0± 2.6	8.5±0.7	86.0±11.1	2.9±0.02
	PV	9.8± 1.7	4.9± 0.3	26.0±1.6	383.0±41.0	50.0± 1.9	15.8±1.2	70.0± 10.0	2.7±0.03
21:	T.c	[a] 7.3±0.9	[a] 3.1±0.1	23.3±1.5	384.0±12.0	59.0±4.4	11.0±1.0	71.3±9.5	2.7±0.03
	PV	14.6±1.5	9.0±1.4	31.0±2.0	403.3± 14.5	53.3±3.5	7.0± 0.6	84.0± 13.0	2.7±0.03
28:	T.c	[a] 32.3±2.7	18.6±1.4	27.0±1.8	412.3±8.9	63.0±3.9	8.6±0.9	71.0± 9.0	3.1±0.05
	PV	[a] 33.3±2.9	16.0±1.2	34.0±2.1	521.7±15.0	81.0±7.1	5.5±0.6	[a] 56.7±7.2	3.4± 0.05

Results expressed as Mean values and SEM; n = 5; [a] P< 0.05; [b] P < 0.001 (ANOVA).
PV represents Proviron; T.c *Tetracarpidium conophorum;* 0.00 controls; ACP, PA, ALP acid, prostatic and alkaline phosphatase respectively; UA uric acid; TP total protein; TCH total cholesterol.

Table 2b.

Group	Time(days)	Treatment Received
8	7	*T.conophorum* (100 mg/kg/day)
9	14	*T.conophorum* (100 mg/kg/day)
10	21	*T.conophorum* (100 mg/kg/day)
11	28	*T.conophorum* (100 mg/kg/day)
12	7	Proviron (25 mg/kg/day)
13	14	Proviron (25 mg/kg/day)
14	21	Proviron (25 mg/kg/day)
15	28	Proviron (25 mg/kg/day)
16	*0.0*	Normal saline (0.5 ml /kg; control)

nephro-protective effects. This is also consistent with the past works (Uche and Obianime, 2008; Obianime and Uche, 2008).

The flavonoids, as an anti-oxidant in this plant may contribute to the effects of this plant as hepatoprotective and nephroprotective, antimicrobial, anti-inflammatory, and anti-carcinogenic effect (Okwu and Josiah, 2008). Alkaloids in this plant may be responsible for its antimicrobial properties (Ajaiyeoba and Fadare, 2006). This is consistent with the past works (Okwu and Josiah, 2008). Alkaloids and their synthetic derivatives are used as basic medicinal agents for their antispasmodic and bactericidal effects .Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membranes (Okwu and Josiah, 2008).

The Uric acid, total cholesterol and protein lowering effect of these plants indicate that the plant can have anti-hypertensive, anti-inflammatory and anti- nociceptive effects. This is also consistent with the past works (Uche and Obianime, 2008; Obianime and Uche, 2008).

In time – dependent study, 100 mg/ kg *T. conophorum* caused different effects on the levels of the biochemical parameters of the male guinea pigs,

after 7, 14, 21, and 28 day post – exposure (Tables 2 and 2b). On comparison of the observed effects of *T. conophorum* on biochemical parameters, with that of 25 mg/kg proviron on the same group of animals, it was found to be similar, although *T. conophorum* showed a more remarkable decrease on the levels of ACP, PA, ALP , UA, Creatinine and TCH on the 21st day.

This study therefore confirms the claimed folkloric use of *T. conophorum* as a hepatoprotective and nephroprotective agent. It also supports the claims on the use of the plant as anti-lipidemic, analgesic and anti-inflammatory and antihypertensive agent. Further studies are on the way to isolate, identify and characterize the active principles in the seeds of *T. conophorum* as well as identification of mechanism of action of *T. conophorum.*

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