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NJBMB/009/12 **SHORT COMMUNICATION**

Effect of methanolic extract of *Tetracarpidium conophorum* (African walnut) seeds on haematological parameters of carbon tetrachloride treated male rats

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ABSTRACT: The effect of the methanolic extract of *Tetracarpidium conophorum* seeds on the haematological parameters of carbon tetrachloride treated male rats was investigated. Thirty two albino rats (170 ±10.0) g were assigned into 8 groups of 4 rats each. Animals in Group I (control) was given distilled water only, while those in groups II, III and IV were administered *T. conophorum* extract at different concentrations of 250, 500, 750 mg/kg body weight daily for two weeks, thereafter, they were given the extract three times per week for three weeks. Group V received CCl_4 only (positive control) at a dose of 0.598g/kg body weight. Groups VI, VII, and VIII animals were given the extract (same doses), for two weeks, then CCl_4 was given orally for two weeks, three times per week simultaneously with the extract. The results showed that CCl_4 only produced significant decrease ($P<0.05$) in white blood cell (WBC count), red blood cell (RBC), hematocrit (HCT), platelets, plasma viscosity (PV), fibrinogen (FB), mean cell volume (MCV) and mean cell hemoglobin (MCH) while there was significant increase ($P>0.05$) in lymphocytes when compared with the control. When the extract was administered alone there was significant increase ($P<0.05$) in white blood cell, lymphocytes, plasma viscosity, and fibrinogen, while platelets were reduced significantly ($P<0.05$) when compared with the control. Furthermore, when the extract was administered to CCl_4 treated rats, there was significant increase ($P<0.05$) in WBC, RBC, HCT, PV and FB while platelets, neutrophils were reduced significantly ($P<0.05$) when compared with the positive control. The results of this study suggest that *T.conophorum* extract could have a potential for the management of haematological disorders and protect blood cells from the damaging effect of CCl_4.

KEYWORDS: Heamatological parameters, carbon tetrachloride, *Tetracarpidium conophorum*

1. Introduction

Carbon tetrachloride (CCl_4) is a haloalkane used in a variety of industrial and chemical applications. It has been widely used for its solvent properties, particularly as a refrigerant, propellant for aerosol cans, dry-cleaning agent in industry, household spot remover, grain fumigant and as an intermediate in the synthesis of chlorofluorocarbons. As a result of its widespread use, CCl_4 is a common contaminant of ground and surface waters where it persists for years. Therefore, CCl_4 is now of greatest concern as an environmental contaminant (ATSDR, 1994; Guo *et al*., 2000).

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Tetracarpidium conophorum (African walnut) belongs to the family of Euphorbiacea and it is commonly known in Southern Nigeria as ukpa (Igbo), in Western Nigeria as awusa or asala (Yoruba) and ọkhue in Bini. It is a perennial woody climber commonly found in low bush especially in Africa, America, Europe and Asia (Hutchinson and Dalziel, 1987). The leaves are globrous, ovate, long and margin toothed. The bases of the leaves are broaded and rounded up to 15 inches with slender petioles that are up to 2 inches long. The fruits are four winged, ridged between wings and up to 3 inches in diameter. African walnut have a bitter taste upon drinking water and can be eaten raw, cooked or with roasted corn (Nuhu *et al*., 2000; Okerulu and Ani, 2001). This plant possesses multiple medicinal properties such as antifertility, antimicrobial, antioxidant

(Ajaiyeoba and Fadare, 2006), anticancer (Herbert *et al*., 1998) and antidiabetic (Kaneto *et al*., 1999).

There is paucity of information on the effect of *T. conophorum* on haematological parameters. Therefore, due to the widespread consumption of walnut seed, this study seeks to determine the effect of the methanolic extract of the seed of *Tetracarpidium conophorum* on some haematological parameters of CCl_4 treated rats.

2. Materials and methods

2.1 Plant material

The seeds of *T. conophorum* used in this study were obtained from open forest at Ovia North East local government area, Edo state, Nigeria. The fresh walnut seeds were identified by Dr M.E Osawaru and authenthecated by Professor MacDonald Idu both of the Department of Plant Biology and Biotechnology of the University of Benin, Benin City, Nigeria. Herbarium specimen (voucher number UBHe0153) was deposited at the herbarium of University of Benin.

2.2 Chemicals

Methanol, CCl_4 (98.8% purity), sodium citrate, calcium chloride were products of Sigma-Aldrich Chemical Company Ltd., St Louis, U.S.A.

2.3 Animals

Thirty two male albino rats weighing 170±10.0 g were purchased from the animal house of the Department of Pharmaceutical Chemistry, University of Benin, Benin, Nigeria. The animals were housed in galvanized rat cages and acclimatized for two weeks on guinea growers mash (Bendel Feed and Flour Mill, Ltd, Ewu, Nigeria) and allowed to drink water freely. The handling of the animals was in accordance with the principle of Laboratory Animal Care (NIH, 1985).

2.4 Preparation of extract

The seeds were rinsed properly, de-shelled, cut into pieces, sundried in the open air until fully dried. The dried samples were then pulverized into powder and stored in an air-tight container. *T. conophorum* seed powder (200 g) was extracted in 2000 mL of absolute methanol at room temperature for 72 hours. The samples were filtered with Whatman No. 50 filter paper and the filtrate evaporated to dryness by a rotary evaporator (RE 300, Bibby Scientific, UK) to give 45.2 g corresponding to a percentage yield of 22.6 %. The resultant yield was stored in an air-tight container and kept in the refrigerator maintained at 4^oC.

2.5 Animal grouping

The rats were divided into eight groups of four rats each:

Group I: Control was given distilled water *ad libitium* and the standard commercial diet, twice a day, for three weeks.

Groups II, III and IV were given 250, 500 and 750 mg/kg body weight of extract orally daily for 2 weeks, then three times per week for 3 weeks respectively.

Group V was given CCl_4 only at a dose of 0.597 g/kg body weight orally.

Groups VI, VII and VII was given 250, 500 and 750 mg/kg body weight of extract orally daily for 2 weeks respectively, then 0.597g/kg body weight of CCl_4 orally for 2 weeks, 3 times per week simultaneously with the extract.

Twenty four hours after the end of the experimental period, rats from both control and experimental groups were sacrificed by cervical dislocation. Blood sample was collected from a prominent artery and directly from the heart into a bottle containing heparin.

2.6 Determination of haematological parameters

Evaluation of the hematological parameters was done using automated hematological analyser K-X 21 made by Sysmex, Kobe Japan.

2.7 Preparation of plasma

Blood sample was collected from albino rats using heparinised bottle. From this, plasma samples were obtained by centrifugation, 3000 x *g* for 10 minutes using an electric centrifuge (SM 80 – 2, Bran Scientific and Instrument Company, England).

2.8 Determination of fibrinogen content

The fibrinogen content was determined by Ingram's clot weight method (Ingram, 1961). When $CaCl_2$ is added to citrated plasma, this triggers off the intrinsic pathway of coagulation resulting in the formation of fibrin clot which can be collected, weighed. Test plasma (1ml) was pipetted into appropriately labeled test tubes in a water bath maintained at 37°C. Thereafter, 1 ml of pre-warmed 0.025M $CaCl_2$ was then added to each tube and mixed. The mixture was left in a water bath for 30 minutes with an applicator stick dipped into each test tube so that fibrin clot formed can be wound around the stick. When all the adherent fibrins were recovered from the applicator stick, it was washed in distilled water twice and then dried by blotting with Whatman no 1 filter paper. Thereafter it was weighed using sensitive balance.

2.9 Determination of plasma viscosity

Plasma viscosity test is based on the comparison between the flow rate of plasma and distilled water under equal pressure and constant temperature through capillary tubes of equal pore and length. The result is expressed as viscosity of plasma relative to that of water.

The plasma to be tested was drawn up; air bubbles were excluded from the vertical syringe until the plunger passes the 1ml mark. The plunger was then completely withdrawn and immediately the lower meniscus fell to the 1.0 ml, a stop watch was started. The time taken for 1.0 ml of the plasma to drop down the syringe was noted. This was done three times on each sample and the mean flow rate was calculated as described by Ried and Ugwu (1987).

The relative plasma viscosity was calculated from the expression:

$$\text{Plasma viscosity} = \frac{\text{Test flow rate}}{\text{Flow rate of distilled water}}$$

2.10 Statistical analysis

Results were expressed as mean of 4 determinations ± SEM. The data were subjected to one-way analysis of variance (ANOVA), and differences between means were determined by Duncan's multiple range tests using the Statistical Analysis System (SPSS Statistics 17.0). P values $\leq$ 0.05 were regarded as significant.

3. Results

Table 1 shows that there was a significant increase ($P<0.05$) in the WBC, lymphocytes and plasma viscosity when the rats were given only the methanolic extract of *T. conophorum* at all doses investigated when compared with the control. In contrast, the extract significantly reduced ($P< 0.05$) platelets, fibrinogen at all the doses investigated when compared with the control. However, when the rats were treated with CCl_4, WBC, PCV and RBC were significantly reduced ($P< 0.05$), but the extract restored the levels of WBC, PCV and RBC back to normal. CCl_4 reduced plasma viscosity, fibrinogen and platelets, but the extract reduced platelet even more than CCl_4. The reduction of neutrophils by the CCl_4 was further reduced by the extract except at 250 mg/kg body weight. However there was a significant increase ($P<0.05$) in lymphocytes, which was maintained by the extract.

4. Discussion

In the present study, the methanolic extract of *T. conophorum* produced varying effects on the haematological parameters of male rats. The increase in white blood count and the differential count could be attributed to stimulation of the immune system response caused by the extract. The findings in the present study agree with that of Yakubu *et al* (2007). Increase in white blood count and differential count could also be used to determine the state of the immune system of an organism. However the medicinal plant did

Table 1: Effect of methanolic extract of *T. conophorum* seeds on some hematological parameters of male rats

Group	Parameters												
	WBC ($X10^3/\mu L$)	**RBC** ($X10^6/\mu L$)	**HGB** (g/dl)	**HCT** (%)	**MCV** (fl)	**MCH** (pg)	**MCHC**(g/dl)	**PLT** ($x10^4/\mu L$)	**LYM** (%)	**MXD** (%)	**NEUT** (%)	**PV**	**FB (g)**
Control (distilled H_2O only)	7.41 ± 0.36	7.79± 0.15	13.75± 0.95	41.60± 2.30	54.45± 1.95	18.00± 0.90	33.05± 0.45	738.00± 57.00	76.70± 3.60	3.50± 0.50	20.3± 2.60	1.47± 0.03	2.05± 0.05
250 mg/kg bwt of extract	8.65 ± 0.35[a]	7.30± 0.67	12.35± 1.75	33.5± 2.10	54.80± 1.00	18.25± 0.55	33.35± 0.45	211.50± 133.50[a]	84.20± 4.40[a]	1.50± 0.50	13.05± 1.65	1.67± 0.02[a]	1.89± 0.01[a]
500 mg/kg bwt of extract	7.76± 0.26[a]	7.22± 0.22	11.85± 1.25	36.15± 5.15	56.45± 0.95	18.6± 1.00	32.95± 0.55	77.00± 15.00[a]	80.85± 1.25[a]	3.95± 2.05	13.00 ±1.10	1.70± 0.05[a]	1.90± 0.10[a]
750 mg/kg bwt of extract	8.15± 0.35[a]	7.37± 0.30	12.65± 0.45	40.80± 2.20	55.35± 0.75	17.20± 0.10	31.05± 0.55	259.00± 49.00[a]	82.35± 0.15[a]	4.50± 1.50	11.85 ±2.05	1.46± 0.01	2.35± 0.05
CCl_4 Only	5.50± 0.70[a]	6.14± 0.43[a]	14.70± 0.40	35.00± 1.00[a]	59.90 ±0.30[a]	9.65± 0.75[a]	31.3± 0.40	357.50 ±52.50[a]	83.35± 3.85[a]	1.30± 0.10	19.90± 0.60[a]	1.08± 0.01[a]	1.65± 0.05[a]
CCl_4 &250 mg/bwt of extract	12.55± 0.55[b]	8.25± 0.01[b]	13.15± 0.65	43.10± 0.40[b]	52.25± 0.55	16.20± 0.50[b]	30.5± 1.20	166.00± 56.00[b]	71.80 ±0.20[b]	5.50± 2.50	21.60 ±3.80	1.58± 0.01[b]	1.95± 0.05[b]
CCl_4 &500mg/kg bwt of extract	10.05± 0.25[b]	7.85±0.99[b]	11.85± 1.05	41.30± 1.10[b]	56.30± 2.40[b]	18.40± 0.95	31.00± 0.40	250.00± 108.00[b]	82.45± 0.95[b]	2.00 ±0.00	16.55± 0.05[b]	1.82± 0.04[b]	1.50± 0.10
CCl_4 &750mg/kg bwt of extract	8.65± 1.15[b]	7.96± 0.49[b]	13.05± 0.95	42.35± 3.65[b]	53.15± 1.35[b]	16.4± 0.20[b]	30.85± 0.45	[b]214.00± 12.00	86.00 ±0.50[b]	8.00± 1.00[b]	14.75± 0.25[b]	1.87± 0.02[b]	2.05± 0.20[b]

Values are expressed as means ±SEM of 4 determinations. [a]Statistically significant at $P<0.05$ compared to the control, [b]statistically significant at $P<0.05$ compared to CCl_4-treated rats;

Legend: WBC; white blood cell count, RBC; red blood cell count, HCT; hematocrit, HGB; hemoglobin, MCH; mean cell hemoglobin, MCV; mean cell volume, MCHC; mean cell hemoglobin concentration, PLT; platelet count, LYM; lymphocyte, MXD%; percentage mixed, NEUT; neutrophils, FB; fibrinogen, PV: plasma viscosity

not affect the red blood cells, hematocrit, mean cell volume and hemoglobin. The decreased platelets following the administration of the different doses of the extract could have a potential for the management of haematological disorders e.g. thrombosis, anemia. Furthermore, the increase in the level of lymphocytes, the main effector cells of the immune system by the extract, may suggest stimulation of the immune system (McKnight *et al*., 1999; Yakubu *et al*., 2007).

Oral administration of CCl_4 greatly affected some haematological parameters; lymphocytes and MCV were significantly increased while WBC count, PCV, platelet, plasma viscosity, fibrinogen and RBC were reduced. The significant reduction in the levels of WBC following the repeated exposure of the rats to CCl_4 could be attributed to neutropenia. This result is similar to the findings of Jirova *et al* (1996) and Mandal *et al* (1998) who stated that exposure to CCl_4 induced significant decrease in leukocyte count in the peripheral blood of mice. Activated neutrophils have been demonstrated to play an essential role in free-radical mediated injury by inducing extracellular release of superoxide and other free radicals (McCord *et al*., 1994),which are toxic to the host cells including neutrophils itself, thereby resulting in their decrease (Jirova *et al*., 1996).

The effect of methanolic extract of *T. conophorum* seeds on haematological parameters of CCl_4 treated rats, as presented in Table 1 shows that WBC, RBC, MCHC were increased whereas MCV and MCH were reduced at different concentrations of 250, 500 and 750 mg/kg body weight of the extract. When the rats were treated with CCl_4 the plasma viscosity decreased. The extract restored plasma viscosity which was decreased by CCl_4. There was also significant decrease in plasma fibrinogen, when the rats were administered carbon tetrachloride. The extract restored plasma fibrinogen that was decreased by CCl_4.

In conclusion, the study has demonstrated that *T. conophorum* extract could have a potential for the management of hematological disorders and protect blood cells from the damaging effects of exposure to CCl_4 and this protection might be attributed to the antioxidant properties of the extract.

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