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COMPARATIVE EFFECTS OF SCOPOLETIN AND CYANIDE ON SERUM ELECTROLYTES, UREA, CREATININE AND SOME HAEMATOLOGICAL PARAMETERS OF RATS

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ABSTRACT

The effects of scopoletin (6-methoxy 7-hydroxycoumarin) and cyanide on serum Na^+, K^+, Urea, creatinine and some haematological parameters of female Wistar rats were compared. The rats were randomly divided into six groups, and administered respectively 7μg/ml, 21μg/ml, 35μg/ml scopoletin, 1.8mg/ml cyanide, 10% dimethyl sulphoxide (the vehicle for the administration of scopoletin) and 1ml distilled water orally per kg body weight at 24 hourly intervals. All the rats were maintained *ad libitum* on chicks' mash and were sacrificed after 14 days. Scopoletin at the level found in processed cassava diet (7μg/ml) increased bleeding time, Rbc count, serum urea and K^+ levels compared to 1.8mg/ml cyanide which is approximately the level consumed by a 70kg man in cassava consuming populations per day. Among the groups administered scopoletin serum levels of K^+ and creatinine increased with increasing concentration while Na^+ and Urea level decreased with increasing concentration. The results of this study suggest further investigations on the effects of scopoletin and cyanide on haematopoiesis and kidney function.

Keywords: Scopoletin, Cyanide, Kidney function, haematopoiesis.

INTRODUCTION

The importance of cassava (*Manihot esculenta* Crantz) as a staple food crop in Africa cannot be over emphasized. Not only does it produce a cheap source of energy for human consumption, it also serves as feed for livestock and has a wide industrial application. Traditionally, cassava tuberous roots are processed by various methods into numerous products utilized in diverse ways according to local customs and preferences (Hahn, 1992). In some cultures, the leaves are also consumed as a favourite green vegetable (Hahn, 1992; Bokanya, 1994).

However, cassava roots easily deteriorate soon after harvest and during storage, producing bluish fluorescent and phenolic components. Scopoletin (6-methoxy 7-hydroxycoumarin), the major fluorescent coumarin compound formed following this deterioration has been identified in processed cassava products and is not significantly affected by post processing treatments such as sun drying, refrigeration, and storage (Obidoa and Obasi, 1991).

Scopoletin is a potent hypotensive and non-specific spasmolytic agent. It has also been identified as an active principle in the traditional infusion of the fruit of *Tetrapleura tetraptera* TAUB in ethnopharmacology of West Africa (Adesina *et al*, 1981; Ojewole and Adesina, 1983). Before the discovery of scopoletin and other anutrients in cassava, all toxic effects of cassava ingestion were attributable to cyanogenic glucosides present in cassava which on hydrolysis yield hydrogen cyanide as one of its products. Hydrogen cyanide acts by preventing the intracellular oxidative processes, although the blood does not lack oxygen.

In this study, the effects of scopoletin and cyanide on plasma electrolytes, creatinine, urea and some haematological parameters of rats were compared.

MATERIALS AND METHODS

Forty-eight (48) female rats (Wistar strain) weighing 95 – 150g got from the Veterinary Research Institute, Vom, Jos, Plateau State, Nigeria were used for this work. The animals were randomly divided into six groups and were administered respectively 7μg/ml scopoletin, 21μg/ml scopoletin, 35μg/ml scopoletin, 1.8mg/ml cyanide, 10% dimethyl sulphoxide (the vehicle for the administration of scopoletin) and 1ml distilled water orally per kg body weight at 24 hourly intervals for 14 days. All the rats were maintained on chicks' mash containing 54% carbohydrate, 10% protein, 2% fat, 20% fibre, 2% normal supplement and 1% vitamin mix. This feed was

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COMPARATIVE EFFECTS OF SCOPOLETIN AND CYANIDE ON SOME SERUM ELECTROLYTES, UREA, CREATININE AND SOME HAEMATOLOGICAL PARAMETERS OF RATS POST 14 DAYS

Treatment		Parameters							
		Na^+ (mMol/l)	K^+ (mMol/l)	Urea (mg/ml)	Creatinine (mg/100ml)	Rbc count ($x10^6$/ml)	Hb (g/100ml)	PCV (%)	Bleeding Time (mins)
Distilled water		147.50 ± 13.58	3.26 ± 0.64	14.43 ± 4.60	1.43 ± 0.01	6.70 ± 0.58	14.58 ± 0.48	44.25 ± 1.32	7.31 ± 0.92
10% DMSO		169.00 ± 14.82	2.95 ± 0.13	11.83 ± 1.01	1.60 ± 0.08	4.72 ± 1.15	15.50 ± 0.22	46.50 ± 0.65	4.62 ± 0.36
Cyanide	1.8mg/ml	180.00 ± 20.55	3.25 ± 0.52	15.55 ± 5.30	1.53 ± 0.12	8.73 ± 0.61	16.33 ± 0.36	49.00 ± 1.08	5.56 ± 0.73
Scopoletin	7µg/ml	174.00 ± 10.52	3.33 ± 0.20	22.00 ± 3.33	1.31 ± 0.26	15.58 ± 0.13	12.25 ± 0.32	45.75 ± 0.94	9.31 ± 1.67
	21µg/ml	148.00 ± 25.86	3.35 ± 0.33	8.86 ± 1.82	1.66 ± 0.08	11.29 ± 0.89	15.91 ± 0.16	47.75 ± 0.48	5.69 ± 1.08
	35µg/ml	136.00 ± 30.01	3.39 ± 0.21	7.40 ± 1.06	1.80 ± 0.10	12.74 ± 3.18	16.25 ± 0.34	48.75 ± 4.03	8.19 ± 0.68

n = 4
Values = mean ± S. E. M

bought from E.C.W.A., Jos, Nigeria. The animals had free access to feed and water throughout the two week duration of the experiment. They were housed under tropical conditions and were exposed to 12 hours light/dark cycle.

Collection of samples: Blood samples were collected from the tail of each rat for the determination of haematological parameters (red blood cells count, haemoglobin concentration and packed cell volume). The rats were then sacrificed by decapitation and blood samples collected in dry centrifuge tubes. An aliquot of blood sample from each of the rats were placed into EDTA bottles for assay of blood urea while the rest were allowed to clot. Centrifugation followed at 3000r.p.m for 10mins and clear serum was collected separately for each sample. The sera were used for the estimation of electrolytes and creatinine.

Determination of bleeding time, Red blood cells (RBC) count, haemoglobin and packed cell volume (PCV) levels: The bleeding time was determined by the time it took for cessation of bleeding, following a slight cut at the tail of the rat. Cessation of bleeding was determined by the filter paper blot procedure (Brown, 1976). Red blood cells count, haemoglobin concentration and packed cell volume (PCV) were determined according to standard procedures described by Coles, (1974) and Schalm *et al*, (1975).

Assay of blood urea and creatinine levels:

Blood urea, and creatinine levels were assayed according to the methods of Koch and McKeekin, (1924) and Henry *et al*, (1972).

Estimation of serum Na^+ and K^+ levels

Levels of serum Na^+ and K^+ in the rats were estimated by flame photometry.

Statistical Analysis:
This was done using the Students' t-test.

RESULTS

Analysis of electrolyte levels (Na^+ and K^+) among the various groups show that the group administered cyanide had the highest serum Na^+ followed by the group on 7µg/ml scopoletin. K^+ level was highest among the group administered 35µg/ml scopoletin. Blood urea level was highest among the group administered 7µg/ml scopoletin. Among the groups administered scopoletin, the serum levels of K^+ and creatinine increased with increasing concentration while Na^+ and urea decreased with increasing scopoletin concentration. Both haemoglobin concentration and PCV increased from below normal values at 7µg/ml scopoletin to slightly above normal at 35µg/ml scopoletin. Generally, scopoletin treatment at the various levels increased Rbc count relative to both distilled water and the solvent (DMSO) controls. Bleeding time was highest at 7µg/ml scopoletin.

DISCUSSION

In this study, Na^+ decreased with increasing concentration of scopoletin. The cyanide treated group had the highest plasma Na^+ level. A lot of factors could lead to a decrease in plasma sodium concentration. These include, gastrointestinal and renal losses, sweating and expansion of extracellular fluid. Hyponatremia may also develop in acute intermittent porphyria and variegate porphyria (Eastham, 1985). In a study in our laboratory, it was observed that rats on scopoletin incorporated diet drank more water than both control rats and those on cyanide incorporated diet (Ezeanyika, Unpublished data). Increased ingestion of

water by rats on scopoletin may have led to increased extracellular fluid volume. There were significant increases ($p<0.5$) in plasma K^+ level in the groups on scopoletin relative to the other groups. Two factors that can account for increased plasma K^+ are impaired excretion by the kidneys and increased cell breakdown accompanying increased protein catabolism (Varley, 1960). In another work carried out alongside this, it was observed that plasma protein decreased as scopoletin increased.

Among the scopoletin treated groups, urea level decreased with increase in scopoletin concentration. Urea content over a period is affected by the amount of dietary proteins and growth (Varley, 1960). However, the rats in this study were fed on the same regimen. When animals are growing, the proteins are used for growth. Rats fed on scopoletin incorporated diet grew less than control and cyanide incorporated diets (Ezeanyika *et al*, 1999).

Serum creatinine level increased with increase in scopoletin level. Creatinine is formed from creatine and diffuses freely throughout the body water. An increase in serum creatinine may be as a result of diminished renal excretion.

Erythrocythaemia was observed in both scopoletin and cyanide treated rats and this was statistically significant ($p<0.05$). Over production of red cells may be a physiological response to a low atmospheric oxygen tension in high altitudes or the need for greater oxygenation for the tissues (Roper, 1978). None of this can account for the erythrocythaemia observed in both scopoletin and cyanide treated rats in this study. The explanation may be found in the idiopathic condition of erythrocythaemia, known as polycythaemia vera (erythraemia). This may result from dehydration or be a compensatory phenomenon to increase the oxygen carrying capacity (Roper, 1978). Sub-lethal doses of cyanide may lead to partial inhibition of cytochrome oxidase and concomitant accumulation of reducing potential as NADH (Philbrick *et al*, 1977). This has a pronounced inhibitory effect on oxygen utilization by the tissues and may necessitate compensatory mechanisms. When various factors stimulate the bone marrow to produce red cells, it becomes greatly hyperplasic and produces far greater than normal quantities. Indeed, even the spleen and occasionally the liver as well, may re-establish their haemopoietic functions long after birth when extreme stimuli persists for prolonged periods of time (Guyton, 1971). Scopoletin may likely act as a stimuli for red cells over production. Red blood cells contain haemoglobin and an increase in red cells is expected to also lead to hyperhaemoglobinaemia.

The results of this study show that scopoletin elicited an increase in bleeding time. An increase in bleeding time may indicate a delayed clotting time. This could be attributed to a delayed synthesis of blood clotting factors in the liver, since synthesis of blood clotting factors are hepatic events. It is possible that the action of scopoletin impacts on Ca^{2+} availability since Ca^{2+} reverses scopoletin induced prolongation of bleeding time (Obasi, 1992). Similar findings were obtained by Obasi *et al*, (1994) who showed that administration of single oral doses of scopoletin (60μg/kg body weight) increased the bleeding time in chicks. The reason for the decrease in bleeding time at 21μg/ml of scopoletin is obscure.

The results of this study show that scopoletin and cyanide may have comparable effects on the observed parameters and that their effects on haematopoiesis and the integrity of the kidney deserves further studies.

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