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Effects of aqueous extract of kuding leaves on cyanide-induced toxicity and packed cell volume (PCV)

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Cyanide poisoning occurs when a living organism is exposed to a compound that produces cyanide ions (CN^-) when dissolved in water. Kuding leaves are Chinese leaves used for weight control. Aqueous extract of kuding leaves was assessed for its effect on cyanide-induced toxicity and packed cell volume (PCV) in Wistar rats. Group A was administered 1% Tween-80 at 5 ml/kg. Group B was not induced but received 400 mg/kg body weight of the extract only, Group C received 3 mg/kg of cyanide without treatment, Group D received 200 mg/kg of the extract, Group E received 300 mg/kg of the extract, and Group F received 400 mg/kg of the extract. PCV was determined using automated haematology analyzer (Mindray-BC-28000). A significant reduction ($p>0.05$) in weight was observed for the group that received only 400 mg/kg body weight of the extract and the group that received only 3 mg/kg body weight of cyanide for two weeks when compared with the control group. The test groups showed a significant reduction ($p<0.05$) in weight after four weeks of administration when compared with the control group. The dose of 3 mg/kg of cyanide and 200 mg/kg of the extract showed 6.4% increase in PCV. The dose of 3 mg/kg of cyanide and 300 mg/kg body weight of the extract showed 3.5% decrease in PCV, while 3 mg/kg of cyanide and 400 mg/kg of the extract showed 31.5% decrease in PCV after four weeks. The results obtained suggest that the aqueous extract of kuding leaves at a dose of 200 mg/kg body weight is effective in healing cyanide-induced toxicity in Wistar rats without reducing their PCV.

Key words: Body weight, cyanide-induced toxicity, Kuding leaves, packed cell volume (PCV), cyanide ion (CN^-).

INTRODUCTION

Medicinal plants have been widely used for the treatment of many ailments due to its minimal or no side effects when compared with the synthetic drugs. Kuding tea is a bitter tea that comes mainly from China. The kuding tea leaves are long, slender and skinny, resembling a nail. Many hype its ability to treat colds and sinus infections as well as high blood pressure and to ease pain. It is also said that the kuding tea can assist in weight reduction. It has also been used to cure the common cold, rhinitis, itching eyes, conjunctival congestion, and headache; it is helpful for digestion and alleviating the adverse effects of alcohol (Li et al., 2011a,b). Today, nearly 20 plants from

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different families with similarities in appearance, flavor, and traditional usage in different areas of China are all named “Kudingcha” (He et al., 2003).

Cyanide poisoning occurs when a living organism is exposed to a compound that produces cyanide ions (CN^-) when dissolved in water. Common poisonous cyanide compounds include hydrogen cyanide gas and the crystalline solids potassium cyanide and sodium cyanide. The cyanide ion halts cellular respiration by inhibiting an enzyme in the mitochondria called cytochrome c oxidase. Cyanide poisoning is a form of histotoxic hypoxia because the cells of an organism are unable to use oxygen, primarily through the inhibition of cytochrome c oxidase. Acute hydrogen cyanide poisoning can result from inhalation of fumes from burning polymer products that use nitriles in their production, such as wool, silk, polyurethane, or vinyl.

If cyanide is inhaled it causes a coma with seizures, apnea, and cardiac arrest, with death following in a matter of minutes. At lower doses, loss of consciousness may be preceded by general weakness, giddiness, headaches, vertigo, confusion, and perceived difficulty in breathing. At the first stages of unconsciousness, breathing is often sufficient or even rapid, although the state of the victim progresses towards a deep coma, sometimes accompanied by pulmonary edema, and finally cardiac arrest. A cherry red skin color may be present as the result of increased venous hemoglobin oxygen saturation. Cyanide does not directly cause cyanosis. A fatal dose for humans can be as low as 1.5 mg/kg body weight (Crampton et al., 1979).

In addition to pesticide and insecticide, cyanide is contained in tobacco smoke, smoke from building fires and some foods, like almonds, apricot kernel, apple seeds, orange seeds, and cassava (also known as yuca or manioc). Vitamin B_{12} in the form of hydroxycobalamin or hydroxocobalamin, may reduce the negative effects of chronic exposure and a deficiency can lead to negative health effects following exposure (Crampton et al., 1979).

Exposure to lower levels of cyanide over a long period (e.g., after use of cassava roots as a primary food source in tropical Africa) results in increased blood cyanide levels, which can result in weakness and a variety of symptoms, including permanent paralysis, nervous lesions (Soto-Blanco et al., 2002a,b, 2008), hypothyroidism (Soto-Blanco et al., 2009), and miscarriages (Soto-Blanco and Górniak, 2004). Other effects include mild liver and kidney damage (Sausa et al., 2002; Manzano et al., 2007).

MATERIALS AND METHODS

Sample preparation

Dried leaves of kuding tea were purchased from Eke Awka Market, Awka, Anambra State, Nigeria. It was identified and authenticated by the Ethnobotanist, Mr. P. O. Ugwuozor in the Department of Botany, Nnamdi Azikiwe University, Awka. The plant material was ground using Corona manual grinding machine.

Sample extraction

The ground sample was extracted by soaking in distilled water for 24 h and then filtered and concentrated at 55°C using Electrical Thermostatic Water Bath (Model No. KJ420).

Experimental animals

Exactly 30 Wistar albino rats weighing between 145 and 150 g were purchased from the Animal House of Chris Farms in Awka, Anambra State and used for this study. They were acclimatized for two weeks prior to the commencement of the experiment. They were kept at room temperature and maintained *ad libitum* on growers mash. The animals were weighed and the initial weight was taken before the commencement of the experiment.

Experimental design

The rats were divided into six groups of 5 rats each: Group A: Normal Control (1% Tween-80, 5 ml/kg); Group B: 400 mg/kg extract only; Group C: 3 mg/kg cyanide only; Group D: 3 mg/kg cyanide and 200 mg/kg extract; Group E: 3 mg/kg cyanide and 300 mg/kg extract; Group F: 3 mg/kg cyanide and 400 mg/kg extract.
The animals were orally administered aqueous extract of kuding leaves for 4 weeks and observed for change in weight and packed cell volume (PCV) every week.

Ethical approval

“Principles of laboratory animal care” were followed. All experiments have been examined and approved by the ethics committee of Nnamdi Azikiwe University, Awka, Nigeria.

Statistical analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) software for windows Version 21 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± Standard Deviation. Statistical analyses of the results were performed using Tukey’s Post-Hoc analysis of variance (ANOVA), the student’s t-test. The limit of significance was set at $p<0.05$.

RESULTS

Determination of the effect of cyanide and aqueous extract of kuding leaves on the weight of rats

The results obtained show that continuous administration of cyanide for four weeks without administration of the extract caused a significant ($p>0.05$) reduction in the weight of the rats. Also, the groups that received the aqueous extract of kuding leaves at a dose of 400 mg/kg body weight showed a significant ($p>0.05$) reduction in their weight after four weeks.

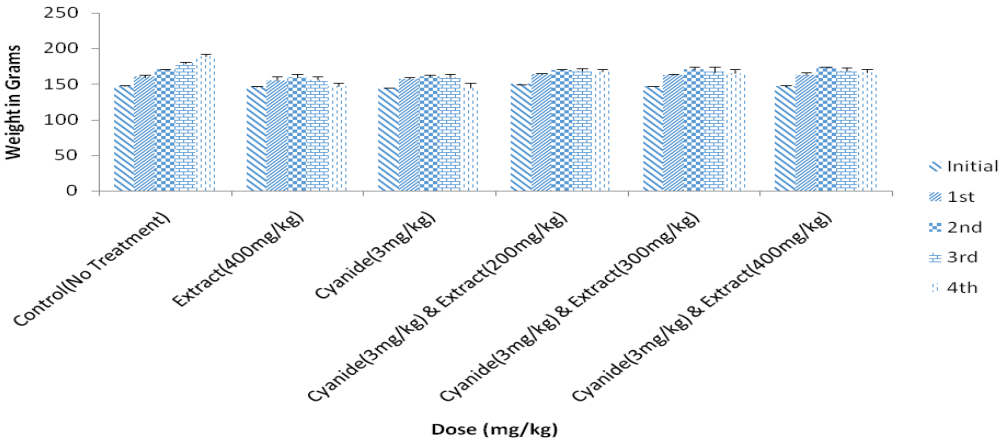

Figure 1. Effects of aqueous extract of kuding leaves on weight when administered at a dose of 200, 300 and 400 mg/kg body weight after administration of cyanide 3 mg/kg body weight done for a period of four weeks.

Determination of the effect of cyanide and aqueous extract of kuding leaves on PCV of rats

Administration of 400 mg/kg body weight of the aqueous extract of kuding leaves showed a significant ($p>0.05$) reduction in PCV when compared with the control group. This reduction was also observed in the group that was administered 3 mg/kg body weight of cyanide only.

DISCUSSION

The observations made showed that the aqueous extract of kuding leaves at certain doses has effect in the weight and PCV of cyanide-induced Wistar albino rats. Oral administration of the extract for the first two weeks after exposure to cyanide toxicity did not reveal any significant ($p>0.05$) decrease in the weight of the rats at different doses (200, 300 and 400 mg/kg) when compared with the control group which was not induced. However, oral administration of 3 mg/kg body weight of cyanide caused a significant ($p>0.05$) reduction in the weight (160.80 ± 2.588) of the rats after two weeks when compared with the weight (170.4 ± 1.140) of the control group. Oral administration of 400 mg/kg body weight of the aqueous extract of kuding leaves without inducing cyanide also caused a significant ($p>0.05$) reduction in the weight (159.2 ± 5.495) of the rats after two weeks when compared with the weight (170.4 ± 1.140) in the control group.

Daily oral administration of the aqueous extract of kuding leaves for three weeks revealed a significant ($p<0.02$) decrease in the weight (168.8 ± 2.863) at a dose of 200 mg/kg. Administration of the extract at a dose of 300 and 400 mg/kg body weight for three weeks revealed a significant ($p<0.05$) reduction in the weight (167.0 ± 7.483 and 168.2 ± 4.658), respectively when compared with the weight (179.4 ± 2.073) of the control group. Continuous administration of the aqueous extract of kuding leaves for four weeks at the doses of 200, 300 and 400 mg/kg body weight showed a significant ($p>0.05$) reduction in the weight (168.0 ± 2.549, 164.8 ± 6.300 and 166.2 ± 5.069), respectively when compared with the weight (188.6 ± 3.361) of the control group.

Daily oral administration of the 400 mg/kg body weight aqueous extract of kuding leaves only without exposure to cyanide for four weeks caused a significant ($p>0.05$) reduction in the PCV of the rats when compared with the control group. Also, administration of cyanide only at a dose of 3 mg/kg body weight caused a significant ($p<0.02$) reduction in the PCV of the rats when compared with the control group that was not induced with cyanide.

Oral administration of 200, 300 and 400 mg/kg body weight aqueous extract of kuding leaves did not cause any significant effect in the PCV of the rats after the first and second week. However, administration of 200 and 300 mg/kg aqueous extract of kuding leaves was found to be effective in restoring the adverse effect of cyanide on PCV after the third and fourth week. This is because the extract at a dose of 200 and 300 mg/kg body weight did not cause a significant ($p>0.05$) reduction in the PCV of the rats for four weeks. The extract at a dose of 400 mg/kg body weight caused a significant ($p>0.05$) reduction in the PCV (22.60 ± 5.549) after the fourth week when compared with the PCV (33.00 ± 2.449) at the start of the experiment. This shows that the administration of the extract at a dose of 400 mg/kg body weight consecutively for periods longer than two weeks after cyanide exposure can be detrimental to the subject.

In conclusion, the aqueous extract of kuding leaves at a dose of 200 and 300 mg/kg body weight can be used to restore the toxic effect of cyanide on the subject by bringing the PCV to normal as shown in Figures 1 and 2. Administration of the extract at a dose of 400 mg/kg body

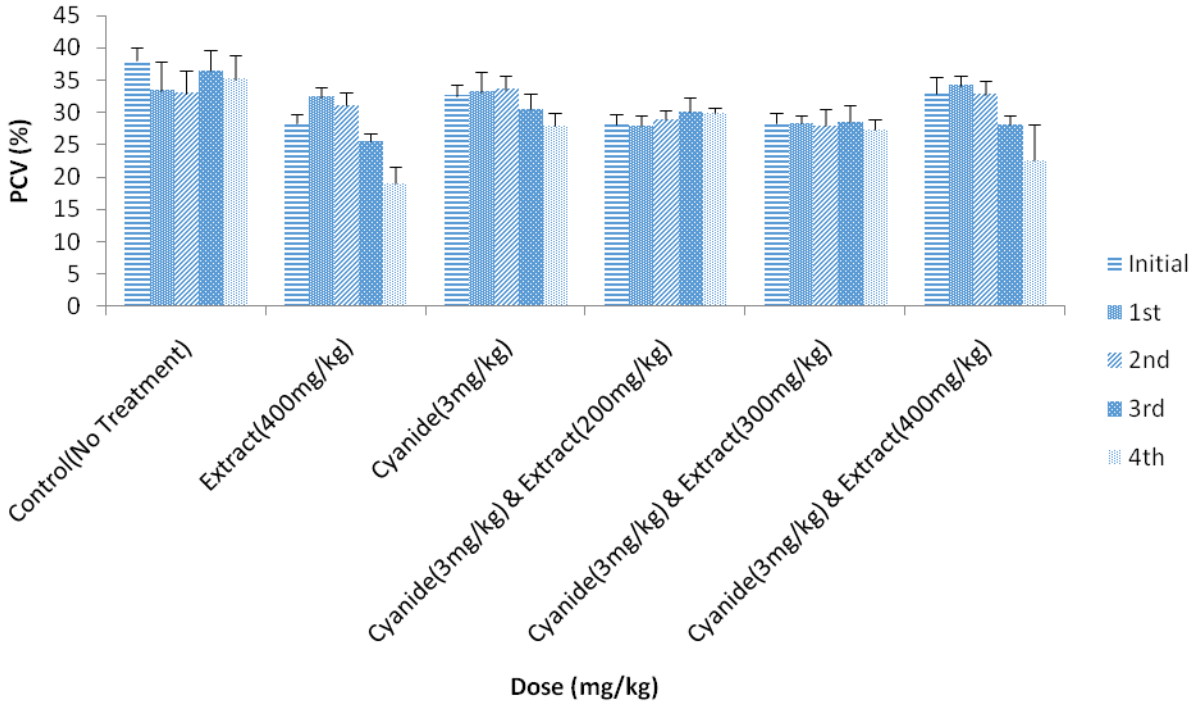

Figure 2. Effect of the administration of aqueous extract of kuding leaves on PCV at different doses after administration of cyanide for a period of four weeks.

weight caused a significant ($p>0.05$) reduction in the PCV showing that the dose is not effective in eliciting a cure because of its adverse effect on PCV.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Crampton RF, Gaunt IF, Harris R (1979). Effects of low cobalamin diet and chronic cyanide toxicity in baboons. Toxicology. 12(3):221-234.

He ZD, Lau KM, But PPH, Jiang RW, Dong H, Ma SC, Fung KP, Ye WC, Sun HD (2003). Antioxidative glycosides from the leaves of *Ligustrum robustum*. Journal of Natural Production 66:851-854.

Li L, Xu LL, Peng Y, He ZD, Shi RB, Xiao PG (2011). Simultaneous determination of five phenylethanoid glycosides in small-leavened Kudingcha from the Ligustrum genus by UPLC/PDA. Food Chemistry. 131:1583-1588.

Li L, Xu LJ, Peng Y, Shi RB, Xiao PG (2011). Comparism of Green tea and four other kind of teas. Journal of Chinese Material Medicine 36:5-10.

Manzano H, de Sousa AB, Soto-Blanco, B (2007). Effects of long-term cyanide ingestion by pigs. Veterinary Research Communication 31(1):93-104.

Soto-Blanco B, Maiorka PC, Gorniak SL (2002a). Effects of long-term low-dose cyanide administration to rats". Ecotoxicology and Environmental Safety 53(1):37-41.

Soto-Blanco B, Maiorka PC, Gorniak SL (2002b). Neuropathologic study of long term cyanide administration to goats". Food and Chemical Toxicology 40 (11):1693-1698.

Soto-Blanco B, Stegelmeier BL, Pfister JA (2008). Comparative effects of prolonged administration of cyanide, thiocyanate and chokecherry (*Prunus virginiana*) to goats. Journal of Applied Toxicology 28(3):356-363.

Soto-Blanco B, Górniak SL (2004). Prenatal toxicity of cyanide in goats-a model for teratological studies in ruminants. Theriogenology. 62(6):1012-1026.

Soto-Blanco B, Pereira FT, de Carvalho AF, Miglino MA, Górniak SL (2009). Fetal and maternal lesions of cyanide dosing to pregnant goats. Small Ruminant Research. 87(1-3):76-80.