Advance Journal of Food Science and Technology 1(1): 39-42, 2009
ISSN: 2042-4876

Submitted Date: September 20, 2009 Accepted Date: October 02, 2009 Published Date: November 10, 2009

Antioxidant Effect of Ethanolic Seed Extract of *Hibiscus sabdariffa linn (Malvaceae)* Alleviate the Toxicity Induced by Chronic Administration of Sodium Nitrate on Some Haematological Parameters in Wistars Rats.

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Abstract: The antioxidant activity of ethanolic seed extract of Hibiscus *sabdariffa l.* was investigated in toxicity induced by chronic administration of sodium nitrate in wistar rats. Twenty-five adult wistar rats were randomly grouped into 5 groups of five rats in each group (n=5). Group I (normal saline); Group II (25mg/kg $NaNO_3$); Group III (25mg/kg $NaNO_3$ + 10mg/kg vitamin C); Group IV (25mg/kg $NaNO_3$ +100mg/kg HS); and Group V (25mg/kg $NaNO_3$ + 200mg/kg HS). All groups received normal saline, Vitamin C and sodium nitrate daily for 60 days respectively. The animals were sacrificed at end of 60 days and blood samples were taken for analysis of total protein and some haematological indices. The toxicity induced by chronic administration of $NaNO_3$ seems to be alleviated by the antioxidant effect Hibiscus *sabdariffa l.* Tocopherol (Vitamin E) and ascorbic acid (Vitamin C) may likely be responsible for the antioxidant effect of Hibiscus *sabdariffa l.* since preliminary phytochemical screening reveal the presences of substantial amount in seed oil. Hibiscus *sabdariffa l.* seed extract are characterized by a very low degree of toxicity with LD_{50} of above 5000 mg/kg in rats.

Keywords: Antioxidant, ethanolic, Sodium nitrate, *Hibiscus sabdariffa* and toxicity

INTRODUCTION

Hibiscus sabdariffa L. is taken as a common local drink popularly known as zobo in Nigeria. It is cultivated for leaf, fleshy calyx, seed or fibre according to the respective properties of the two major varieties *var. ruber* (red) and *var. intermedius* (green) (Dalziel 1973). The thick red and fleshy cup-shaped calyces of the flower are consumed worldwide as a cold beverage and as a hot drink (sour tea) (Morton, 1987). It is known as roselle or red sorrel (English), karkade (Arabic), yakuwa (Hausa), amukan (Yoruba) and okworo ozo (Ibo). *Hibiscus sabdariffa Linn* is a herb belonging to the malvaceae family and it is grown in Central and West Africa, South East Asia, and elsewhere in parts of West Indies, Jamaica and Central America. In Africa roselle are frequently cooked as a side dish eaten with pulverized peanuts. For stewing as sauce or filling for tarts or pies, the products were indistinguishable from cranberry sauce in taste and appearance (Morton, 1987; Olaleye, 2007).

Hibiscus sabdariffa l. is also used in folk medicine against many complaints that include high blood pressure, liver diseases and fever (Dalziel, 1973; Wang *et al*., 2000; Odigie *et al*., 2003; Ali *et al.,* 2005). In Nigeria, a decoction of the seeds is given to augment or induce lactation in poor letdown and maternal mortality (Okasha *et al.,* 2008). *Hibiscus sabdariffa* being a potential herb used as source of many foods and beverages in especially local community in Africa and parts of the World, so its practical benefits needs to be established. In light of this, the study is designed to evaluate the antioxidant effect of *Hibiscus sabdariffa* seed extract.

MATERIALS AND METHODS

Chemicals and drugs: All chemicals and drugs used were of analytical grade. Sodium nitrate, ethanol, (Aldrich Chemical Company, Gillingham England) was obtained from Department of pharmacology Ahmadu Bello University Zaria, Nigeria. Vitamin C (Em – vitamin C, 100mg tabs. Emzor Pharmaceutical Industries, Lagos, Nigeria)

Plant materials: The samples of *Hibiscus sabdariffa* L. seeds were collected in December 2007 in Gaya Hong Local Government in Adamawa state of Nigeria. The plant was identified in the Department of Biological Sciences, Ahmadu Bello University, Zaria and authenticated voucher samples were deposited in the Herbarium section (code number 1056).

Extract preparation: The Hibiscus *sabdariffa l.* seeds were washed thoroughly, sun dried and ground into powder. The extraction of *Hibiscus sabdariffa l.* seeds

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was done using fifty grams (50g) of the powder seeds in soxhlet extractor with ethanol in Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria. The recycling of solvent (ethanol) was allowed to be repeated for complete extraction. The oil extracts were then poured into evaporating dish to evaporate the solvent in the extract over the water bath at the temperature of 40ºC - 45 ºC (Abdul, 1990) and a yield of 17ml of crude extract oil was obtained.

Experimental design: Twenty five wistar rats were used and they were randomly divided into five groups of five rats in each group (n=5). Group I control received normal saline; Group II received 25mg/kg $NaNO_3$; Group III received 25mg/kg $NaNO_3$ + 10mg/kg vitamin C; Group IV received 25mg/kg $NaNO_3$ + 100mg/kg *Hibiscus sabdariffa*; Group V received 25mg/kg $NaNO_3$ + 200mg/kg *Hibiscus sabdariffa*; sodium nitrate was administered chronically to induce toxicity and vitamin C and *Hibiscus sabdariffa* seed extract to treat the toxicity (Isyaku *et al.,* 2009). All the groups were administered normal saline, sodium nitrate, vitamin C and *Hibiscus sabdariffa l.* seed extract for sixty days respectively.

Phytochemical Analysis: The ethanolic seed extract of Hibiscus *sabdariffa l.* were subjected to preliminary phytochemical screening to identify the chemical constituents. The methods of analysis employed were those described by Brain and Turner (1975).

Acute toxicity study: The lethal dose (LD_{50}) of the plant extract was determined by the method of Lorke (1983) using 13 rats. In the first phase rats were divided into 3 groups of 3 rats each and were treated with the ethanolic extract of the seed at doses of 10, 100 and 1000 mg/kg body weight intraperitoneal. They were observed for 24 h for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were also treated with the aqueous extract at doses of 1000, 1600, 2900 and 5000 mg/kg bodyweight *(i. p)*. The median lethal dose (LD_{50}) was calculated using the second phase.

Statistical Analysis: All data are expressed as Mean ± S.E.M. The data obtained were analyzed using one way analysis of variance (ANOVA) and Turkey-Kramer *post hoc* test for multiple comparisons. The ($P<0.05$) will be accepted as significant (Betty and Jonathan, 2003).

RESULTS

Acute toxicity study (LD_{50}): The seed extracts are characterized by a very low degree of toxicity. The acute toxicity LD_{50} of *Hibiscus sabdariffa l.* ethanolic seed extract in albino rats was found to be above 5000 $mgkg^{-1}$ according to the method of Lorke (1983).

Phytochemical Analysis: The preliminary phytochemical screening of the ethanolic seed extract of *Hibiscus sabdariffa l.* found the presence of alkaloids, saponins, Cardenolides, Deoxy sugar, tannins, steroidal rings, cardiac glycosides, flavonoids and anthraquinones.

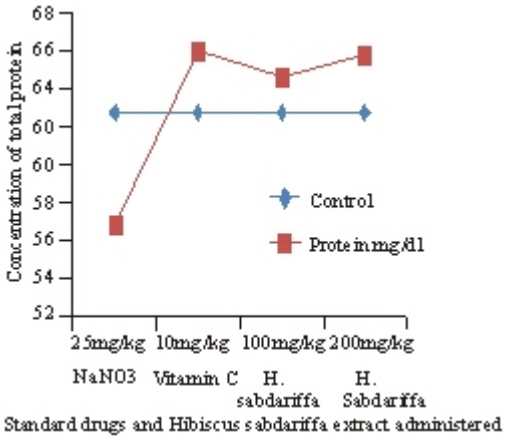

Fig.1: Showing the serum total protein level in control, standard drug and *Hibiscus sabdariffa* extract-treated groups in toxicity induced by chronic administration sodium nitrate.

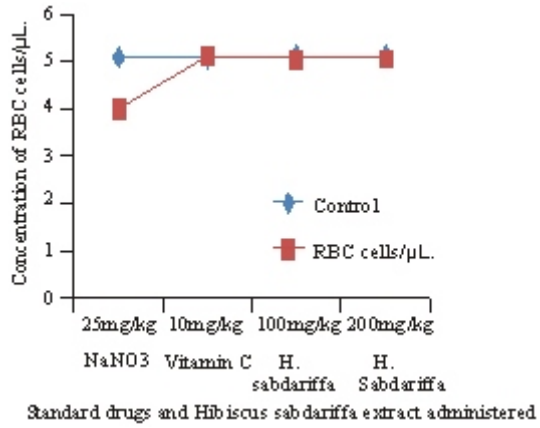

Fig.2: Showing the red blood cell count in control, standard drug and *Hibiscus sabdariffa* extract-treated groups in toxicity induced by chronic administration sodium nitrate

Haematological values: In Fig.1 the results of the total protein in $NaNO_3$ induced toxicity showed a significant decrease ($P<0.05$) when compared to control group while the standard drug (Vitamin C) and Hibiscus *sabdariffa l.* seed extract increases the total protein by alleviating the $NaNO_3$ induced toxicity to the level of control group. In Fig 2, 3 and 4 the haematological indices in $NaNO_3$ induced toxicity showed a significant decrease ($P<0.05$) when compared to control group while Vitamin C and seed extract of *Hibiscus sabdariffa* reverses toxicity induced by $NaNO_3$ by alleviating the haematological indices to the level of control group. The deleterious effect of the $NaNO_3$ induced toxicity seems did not have

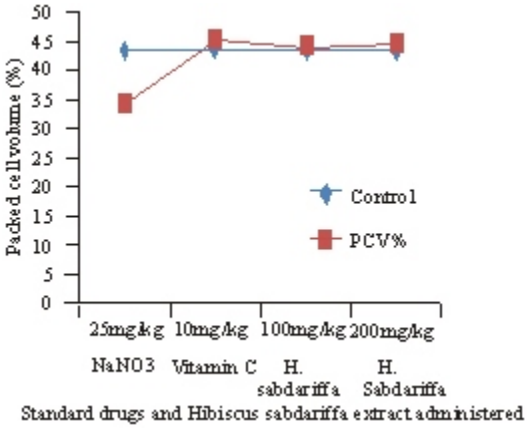

Fig.3: Showing the packed cell volume in control, standard drug and *Hibiscus sabdariffa* extract-treated groups in toxicity induced by chronic administration sodium nitrate

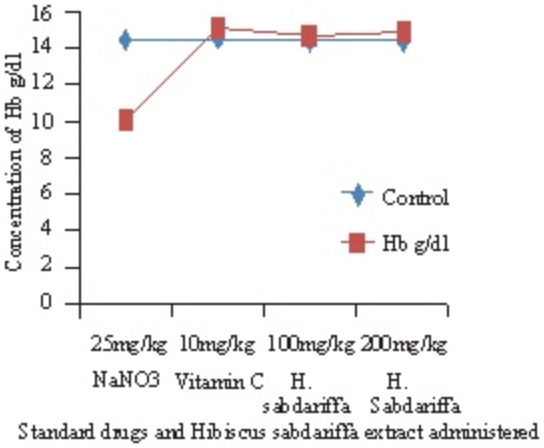

Fig.4: Showing the hemoglobin level in control, standard drug and *Hibiscus sabdariffa* extract-treated groups in toxicity induced by chronic administration sodium nitrate

much effect on the immune, because $NaNO_3$ induced toxicity when compared with control, Vitamin C and *Hibiscus sabdariffa* treated-groups showed no significant statistical decrease ($P>0.05$) in Fig.5 total white blood cell count.

DISCUSSION

The results of the present study showed that, the ethanolic seed extract of Hibiscus *sabdariffa l.* exhibited an antioxidant effect on some haematological indices in alleviating the toxicity induced by chronic administration of sodium nitrate to the level of control and standard drug (Vitamin C) groups. The result is in conformity with Vitamin C and E has ameliorative effect on sodium

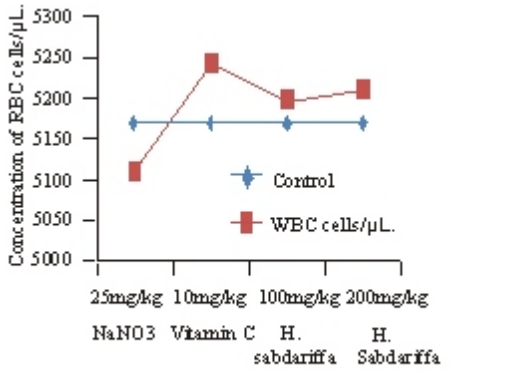

Fig.5: Showing the white blood cells count in control, standard drug and *Hibiscus sabdariffa* extract-treated groups in toxicity induced by chronic administration sodium nitrate.

nitrate-induced toxicity by increasing the reduced sperm count back to normal (Isyaku *et al.,* 2009). The haematological indices in $NaNO_3$ induced toxicity in Fig. 2, 3 and 4 showed a significant decrease ($P<0.05$) when compared to control group while Vitamin C and seed extract of *Hibiscus sabdariffa* reverses toxicity induced by $NaNO_3$ by alleviating the haematological indices to the level of control group. The antioxidant effect of *Hibiscus sabdariffa* may due to the presence of ascorbic acid (Vitamin C) and tocopherol (Vitamin E) as reveal by the preliminary phytochemical screening. Hibiscus *sabdariffa l.* is one of those plants whose different parts phytochemical screening revealed that its calyces have antioxidant activity and as well as its seeds revealed to be a good source of lipid soluble antioxidants, particularly y-tocopherol. Total tocopherol were detected at an average concentration of 2000mg/kg, including α-tocopherol 25%, y-tocopherol 74.5% and δ-tocopherol 0.5%, while the oil of this plant belongs to the linoleic/oleic category; the global characteristics of *Hibiscus sabdariffa* oil suggests that it could have important industrial applications (Mohamed *et al.,* 2007; Abuharfiel *et al.*, 2001). The acute and sub-chronic toxicity studies characterize the plant to have low toxicity which makes it safe for human consumption (Okasha *et al.,* 2008). The toxicity induced by chronic administration of $NaNO_3$ seems to be alleviated by the antioxidant effect of Tocopherol (Vitamin E) and ascorbic acid (Vitamin C) which are present in substantial amount in seed oil of *Hibiscus sabdariffa l.*

ACKNOWLEDGMENT

The authors are grateful to the following; Mohammad Nura of the Department of Human physiology Ahmadu Bello University, Zaria for the role he played in the laboratory during this research. We also acknowledge

mcArthur foundation Ahmadu Bello University, Zaria and University Board of Research of Ahmadu Bello University, Zaria for supporting this research work.

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