NIGERIAN VETERINARY JOURNAL

ISSN 0331-3026

Nig. Vet. J., September 2016 **Vol. 37 (3): 148-154.**

SHORT COMMUNICATION

In Vivo and *In* Vitro Antioxidant Effects of *Icacina trichantha* Tuber Extract

Onakpa, M. M.[1]; Ode, J. O.[1]; Ajagbonna, O. P.[1] and Asuzu, I. U.[2]

[1]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Abuja, P.M.B.117, Abuja. Nigeria; [2] Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. *Corresponding author: Email: onakpamm@yahoo.com; Tel No:+2348033865430.

SUMMARY

The phytochemical and antioxidant properties of the methanol extract of *Icacina trichantha* tuber were evaluated using standard assays. The antioxidant potential was studied with both *in vitro* and *in vivo* models. The *in vitro* procedures involved the use of 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH) and ferric reducing/antioxidant power (FRAP) spectrophotometrically. The *in vivo* models quantified the gradation in the levels of malondialdehyde (MDA), serum superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) activities in rats that were fed on graded doses of the extract incorporated in feed for 12 weeks. DPPH assay revealed that the crude extract possessed a high antioxidant capacity of 67.3% compared to 80.3% with ascorbic acid at 400 µg/ml. The findings from the FRAP test buttressed the fact that the extract displayed a remarkably high and dose-dependent antioxidant effect with a maximal value of 6.7 µM at 800 µg/ml. The extract also exhibited potent *in vivo* antioxidant effects when the plasma MDA levels became depressed while the serum SOD, CAT and GSH values of test rats that received the medium and high dose (0.5 and 1.0 g/kg feed) of the extract were significantly ($p<0.05$) elevated from days 60-90 compared to values in the control and low dose (0.25 g/kg feed) of the extract. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, carbohydrates, reducing sugars, sterols and terpenes in the crude methanol extract of *I. trichantha* tuber. The results of the study demonstrated that *I. trichantha* tuber extract possessed high *in vitro* and *in vivo* antioxidant activities. The extract could be a potential source for the isolation of novel plant-derived antioxidant agents.

Key words: Antioxidant, *Icacina trichantha*, Free radicals, Bioassay.

INTRODUCTION

Reactive oxygen species (ROS) are a group of highly reactive molecules due to the presence of unpaired electron in their structures; featuring prominently among them are superoxide anion, hydrogen peroxide (H_2O_2), peroxyl (ROO-) radicals, and reactive hydroxyl (OH-) radicals as well as the nitrogen derived free radicals: nitric

oxide (NO_2) and peroxynitrite anion (ONOO-) (Cook and Samman, 1996). Free radicals are chemically unstable atoms or molecules that cause damage to cells as a result of imbalance in the value generated within the body and the action of antioxidant defense mechanisms. ROS are also produced during normal aerobic cellular respiration (Ou, 2002). Oxidative stress however, is a deviation from equilibrium where excessive quantities of reactive oxygen and/or nitrogen species overwhelm the endogenous anti oxidative capability of body tissues. The free radicals become unhindered in their ability to cause oxidation of macromolecules, such as proteins, enzymes, lipids and DNA (Dai and Mumper, 2010) to initiate or exacerbate various diseases.

Antioxidants have been detected in a number of products such as cereals, fruits, vegetables and oil seeds (Netzel *et al*., 2007). Antioxidants do not completely get rid of free radicals in the body but retard or minimize the damage caused (Trouillas *et al.,* 2003). The body is therefore in need of sources of antioxidants. Antioxidants are increasingly needed as part of our routine diet and to complement therapeutic interventions in several ailments. Thus, it becomes imperative to explore the plant kingdom for potent antioxidants from natural sources.

Icacina trichantha Oliv. (Icacinaceae) is popularly employed as a traditional herbal medicine in different cultures within Nigeria and West Africa. The plant is locally known as "Urumbia" or "Eriagbo" (referring to its emetic effect) among the *Ibos* of Nigeria, or "Gbegbe" (meaning to cleanse) by the *Yorubas* of Western Nigeria who prescribe it as remedy for food poisoning, constipation, and malaria (Asuzu and Abubakar, 1995). The plant is also acclaimed to serve as a household medicine for emergency uses as virtually every household in some parts of Nigeria preserve a portion of the macerated tuber in *dry gin* as a first-aid medicine.

Recent studies on *I. trichantha* revealed its anti-hyperglycemic effects in alloxan-induced diabetic rats (Onakpa and Asuzu, 2013); the antibacterial, antifungal, sedative, analgesic properties, and protective activities of the extract against leptazole-induced convulsive tendencies have also been reported (Asuzu and Abubakar, 1995). Phytochemical analysis showed the presence of diterpenes and diterpene alkaloids in the extracts (Asuzu and Abubakar, 1995; Onakpa *et al*., 2014). The present study was designed to evaluate the *in vitro* and *in vivo* antioxidant properties of the methanol extract of *I. trichantha* tuber in rats following a prolonged exposure to the extract.

MATERIALS AND METHODS

Experimental Animals

Adult wistar albino rats (180-250 g) of both sexes procured from the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were used for the experiment. The rats were kept under standard environmental conditions of temperature (25^oC), relative humidity (45% – 55%), dark/light cycle of 12 hours. They were fed with standard feed pellets (Feed Masters®) and tap water was provided *ad libitum*. The animals were acclimatized for two weeks prior to commencement of the study. A standard protocol was drawn up in accordance with the Good Laboratory Practice (GLP) Regulations of the WHO (1998) while principles of Laboratory Animal Care (NIH Publication #85-23, 1985) were strictly adhered to in this study.

Plant Collection and Extract Preparation

Fresh tubers of *I. trichantha* were collected from Orba in Udenu Local Government Area of Enugu State, Nigeria. The tubers were identified by Mr. Ozioko Alfred, a

botanist with Bio-resources Development and Conservation Program (BDCP), Nsukka and Omotoso Oloroede, a Professor of Botany, University of Abuja, Nigeria; the voucher specimen, (UNABJ/FVM 456) was deposited in the Pharmacology Laboratory, University of Abuja for reference purposes.

The tubers were sliced, dried under mild sunlight, pulverized into coarse powder and then reduced to finely ground particles in a laboratory hammer mill. Cold extraction was performed on 1.5 kg of the plant material by percolation using 80% methanol to obtain an extract which was filtered using Whatman No. 1 filter paper. The filtrate was concentrated *in vacuo* to dryness using a rotary evaporator connected to a cold water circulator and a pressure pump at 40^{o}C and 210 milibar. The crude *I. trichantha* extract, labeled ITTE was kept at 4^{o}C in a refrigerator for further use in the investigations.

***In vitro* Antioxidant Assays**

DPPH free radical scavanging assay

The method of Mensor *et al*., (2001) was adopted for 2,2-Diphenyl-2-Picrylhydrazyl free radical (DPPH) antioxidant assay. DPPH is a stable free radical, mixing it with a substance that can donate a hydrogen atom gives rise to the reduced form with loss of the violet colour which is monitored at 520 nm (Molyneux, 2004). Ascorbic acid was used as reference standard. The percentage antioxidant activity was calculated as accordingly:

% antioxidant activity [AA] = 100 – ([absorbance of sample-absorbance of blank] X 100)/Absorbance of control.

Ferric Reducing Antioxidant Power (FRAP) Assay

This was done using the method described by Benzie and Strain (1999). When the ferric tripyridyltriazine (Fe^{III}–TPTZ) complex is reduced to the ferrous (Fe^{II}) form, an intense blue colour with an absorption maximum at 593 nm develops.

***In vivo* antioxidant tests**

Lipid peroxidation assay: The study was done as part of a long term (12 weeks) effect of exposure of experimental rats to *I. trichantha* tuber extract. The rats were given the extract at various doses in feed while clean drinking water was provided *ad libitum.* Fifty six (56) albino rats of both sexes were separated randomly into 4 groups (A-D) of 14 rats per group. Group A (control) received feed without extract, B was given 0.25 g extract /kg feed (low dose), C fed on 0.5 g extract/kg feed (medium dose) while group D was provided with 1.0 g extract/kg feed (High dose). The experimental rats were fed in accordance with the normal feed consumption of rats at 10 g feed/100 g b.w./day (Hafez, 1970). Each rat was weighed on a weekly basis as a determinant for the quantity of feed to be taken for the week. Blood samples were collected from the media canthus of the eye of four rats in each group on days 30, 60 and 90 to determine changes in lipid peroxidation.

Lipid peroxidation assay was performed using the method of Buege and Aust (1978) on plasma samples from rats that were given graded extract doses (0.25, 0.5 and 1.0 g extract/kg feed) to assess the mean gradation in malondialdehyde concentration in each animal group by extrapolation from a standard curve generated by the hydrolysis of 1,1,3,3-tetraethoxypropane. Thiobarbituric acid (TBA) reacting substance, malondialdehyde which is a product of lipid peroxidation reacts with barbituric acid to give a red or pink coloration which absorbs maximally at 532 nm (Walling *et al.*, 1993).

Estimation of Superoxide Dismutase (SOD) activity: The concentration of SOD in the rat serum was determined as described by Xin *et al*. (1991). SOD enzyme alternately catalyses the dismutation or partitioning of the superoxide (O_2^-) radical into molecular oxygen (O_2) or hydrogen peroxide (H_2O_2).

Assay of catalase (CAT) activity: Catalase activity was measured according to the method of Aebi (1984). The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. The enzyme activity was expressed in mL^{-1} protein.

*Assay of reduced glutathione (GSH) concentration***:** Reduced glutathione was determined by the method of Ellman (1959). Glutathione reduces disulfide bonds formed within cytoplasmic proteins to cysteines by serving as an electron donor. Once oxidised, glutathione can be reduced back by glutathione reductase, using NADPH as an electron donor (Couto *et al.*, 2013).

*Phytochemical analysis***:** The spot tests for identifying active principles were carried out using the method of Trease and Evans (1989). The crude extract of *I. trichantha* tuber was analysed for the presence of alkaloids, flavonoids, tannins, saponins, carbohydrates, reducing sugars as well as sterols and terpenes following standard techniques. An equal volume of distilled water in a second test tube was used as the negative control for each of the observation. All tests were carried out in triplicates.

Statistical Analysis

Data collected were statistically analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range post-hoc test; mean differences at $p<0.05$ were considered significant.

RESULTS

DPPH spectrophotometric assay

The crude extract of *I. trichantha* tuber demonstrated appreciable antioxidant activity with DPPH radical scavenging method. At the lowest test concentration of 10 µg/ml, the extract exhibited a mean antioxidant activity of 50.4% compared to ascorbic acid which produced 74.3%. In the same manner, the extract elicited 67.3% antioxidant activity at the highest test concentration (400 µg/ml) relative to 80.3% with ascorbic acid (Figure 1).

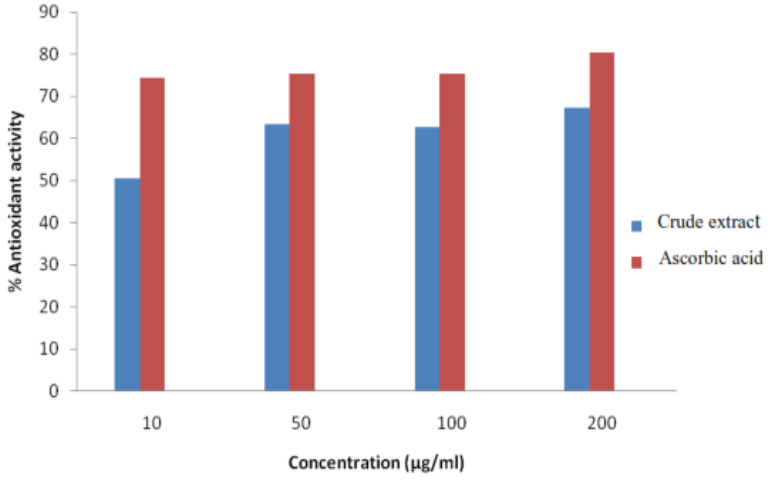


Figure 1: The antioxidant activity of *I. trichantha* tuber extract compared with that of ascorbic acid in DPPH spectrophotometric assay

FRAP Assay with *I. trichantha* tuber extract

At 10 µg/ml, the mean antioxidant power (FRAP value) was 0.43 µM. This increased to 2.0 µM at 100 µg/ml, 3.5 µM at 400 µg/ml and then 6.7 µM at 800 µg/ml (Table I).

***In vivo* Antioxidant assays**

*Superoxide dismutase (SOD) Assay***:** Serum concentration of SOD in rats given medium and high dose (0.5 and 1.0 g/kg feed) of the extract was

TABLE I: FRAP assay with *I. trichantha* tuber extract

Concentration (µg/ml)	Mean change in absorbance between 0-4 minutes	Antioxidant power (FRAP) in µM ± SE
10	0.043	0.43 ± 0.012
50	0.097	0.98 ± 0.003
100	0.197	2.0 ± 0.007[a]
200	0.256	2.6 ± 0.004[a]
400	0.345	3.5 ± 0.023[a]
600	0.453	4.5 ± 0.004[b]
800	0.673	6.7 ± 0.032[c]

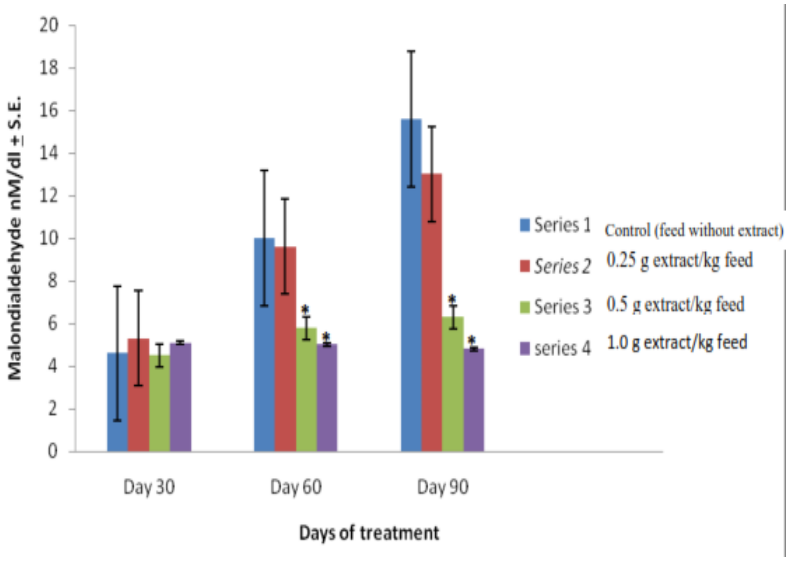
[abc]Significant differences at p<0.05

TABLE II: Effect of long term (90 days) intake of *I. trichantha* tuber extract on superoxide dismutase (SOD) levels of rats

		Mean SOD activity ± SEM (IU/L)		
Group	Treatment	Day 30	Day 60	Day 90
I	Control (feed without extract)	21.6 ± 2	27.3 ± 2	28.6 ± 3
II	ITTE (0.25 g/kg of feed)	23.3 ± 2	28.6 ± 3	29.0 ± 4
III	ITTE (0.5 g/kg of feed)	23.6 ± 1	39.0 ± 1*	45.3 ± 2*
IV	ITTE (1 g/kg of feed)	22.9 ± 3	40.0 ± 5*	50.6 ± 4*

*Significant differences at p < 0.05 when compared to control; ITTE= *I. trichantha* tuber extract

significantly (p<0.05) elevated compared to the control and low dose of the extract from day 60 to the termination of the experiment at day 90. At day 60, control SOD was 27.3 ± 2 when 0.5 g extract/kg feed induced SOD value of 39.0 ± 1 and 1.0 g extract/kg feed produced SOD level of 40.0±4 IU/L. Similarly, at day 90, SOD value in control rats was 28.6 ±3 but 0.5 g extract/kg feed enhanced SOD value to 45.3 ±2 IU/L while 1.0 g extract/kg feed increased the rat enzyme concentration to 50.6±4 IU/L (Table II).



*****Significant at p<0.05 compared to control

Figure 2: Effect of *I. trichantha* tuber extract on MDA levels of rats fed for 90 days

Malondialdehyde (MDA) level in rats on long term intake of *I. trichantha* tuber extract:

The extract of *I. trichantha* tuber induced a significant (p<0.05) decrease in the respective plasma MDA values of test rats that received the medium and high doses (0.5 and 1.0 g /kg feed) of the extract from days 60-90 compared to values in the control and low dose (0.25 g/kg feed) of the

extract. At day 60, the medium dose of the extract (0.5 g/kg feed) decreased the MDA level of 10.0±3 nM/dl observed in control rats to 5.8±4 nM/dl while the high test dose (1.0 g/kg feed) of the extract further reduced the MDA value to 5.0±3 nM/dl. In the same manner, the medium and high dose extract significantly ($p<0.05$) decreased MDA plasma level (15.6±0.5 nM/dl) of rats in the control group to 6.3±5 and 4.8±2 nM/dl respectively. However, within the period of 30 days, enough stress may not have developed in the experimental rats; hence there were no significant ($p>0.05$) differences in MDA values between the control and the treated rats (Figure 2).

DISCUSSION

The crude methanol extract of *I. trichantha* tuber demonstrated a radical scavenging ability of 67.3% relative tp 80% with ascorbic acid, a reference antioxidant at 400 μg/m in the DPPH assay. The extract also produced a dose-dependent antioxidant capacity with a maximal FRAP value of 6.7 μM at 800 μg/ml compared to ascorbic acid, which is known to have a standard FRAP value of 2.0 μM at 1000 μg/ml (Benzie and Strain, 1999). The remarkably high antioxidant activity of the crude extract in relation to ascorbic acid as revealed in the DPPH assay was confirmed in the FRAP test value of 6.7 μM at 800 μg/ml. Any crude compound with an obviously higher percentage bioactivity than a reference, pure agent should no doubt awaken the interest of researchers for further evaluation.

In conclusion, *Icacina trichantha* tuber extract demonstrated potent *in vitro* and *in vivo* antioxidant activities in support of its folkloric use as remedy for several diseases. This assertion was proven when DPPH and FRAP spectrophotometric assays revealed a high antioxidant capacity of the extract which was also collaborated with a reduction in lipid peroxidation but enhanced SOD, CAT and GSH activities in test rats. The tuber extract could be a potential source for the isolation of novel antioxidants.

REFERENCES

AEBI, H. (1984). Catalase *in vitro. Methods in Enzymol*. 105: 121-126.

ASUZU I. U, AND ABUBAKAR I. I. The effects of *Icacina trichantha* tuber extract on the nervous system. *Phytotherapy Research* 1995; 9, 21-25.

BENZIE, I. F. F., and STRAIN, J. J. (1999). Ferric reducing antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In: *Methods in Enzymology*. Vol 299 pp 15-27.

BUEGE, J. A. AND AUST, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymol.* 52: 302-310.

COOK N. C, and SAMMAN S. (1996) Flavonoids-chemistry, metabolism, cardio-protective effects, and dietary sources. *Nutritional Biochemistry*. 7: 66-76.

COUTO, N., MALYS, N., GASKELL, S. AND BARBER, J. (2013). Partition and Turnover of Glutathione Reductase from Saccharomyces cerevisiae: a Proteomic Approach. *Journal of Proteome Research* 12 (6): 2885-94.

DAI, J. and MUMPER, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10): p. 7313-7352.

ELLMAN, G. L. (1959). Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82: 70-77.

Good Laboratory Practice (GLP) Regulation, WHO, 1998

HAFEZ, E. S. E. (1970). Reproductive and breeding techniques for laboratory animals. Lea Fabiger, Philadelphia.

pp. 10-31.

MENSOR, L,I., MENEZES, F.S., LEITAO, G.G., REIS, A.O., DOS, S., COUBE, C.S., and LEITAO, S.G. (2001). Screening of Brazilian plants extract for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*. 15:127-130.

MOLYNEUX, P. (2004). The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology* 26 (2): 212-219.

NETZEL, M., NETZEL, G., TIAN, Q., SCHWARTZ, S., and KONCZAK, I. (2007) Native austraian foods–a novel source of antioxidants for food. *Innovative Food Science and Emerging Technologies*, 8:339–346.

ONAKPA M. M. AND ASUZU I. U. (2013). Histological changes and antidiabetic activities of *Icacina trichantha* tuber extract in beta-cells of alloxan induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine*. 3(8): 628-633.

ONAKPA M. M., MING ZHAO, TANJA GÖDECKE, WEI-LUN CHEN, CHUN-TAO CHE, BERNARD D. SANTARSIERO, STEVEN M. SWANSON AND ASUZU ISAAC UZOMA (2014). Cytotoxic (9*β*H)-Pimarane and (9*β*H)-17-Norpimarane Diterpenes from the tuber of *Icacina trichantha*. *Chemistry and Biodiversity* vol. 11 (12): 1914-1922.

OU, B. (2002) Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *Journal of Agricultural and Food Chemistry*, 50(11): 3122- 3128.

TROUILLAS, P., CALLISTE, C.A., ALLAIS, D.P., SIMON, A., MARFACK, A., DELAGE, C. and DUROUX, J.L. (2003). Antioxidant,anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the limousine countryside as herbal teas. *Food Chemistry*. 80: 399-407.

WALLING, B; ROSENGREEN, B; SHETZER, H. G. and CAMEJO, G. (1993). Lipid peroxidation and measurement of Thiobarbituric Acid Reacting Substances (TBARS) formation in a single microtitre plate, its use for evaluation of antioxidants. *Annals of Biochemistry*, 218: 10-15.

WHO (1980). Expert Committee on *diabetes mellitus*. Second Report, Technical report series, 646. World Health Organization. Geneva.

XIN, Z., WATERMAN, D.F., HENKEN, R.M., and HARMON, R.J. (1991). Effects of copper status on neutrophil function, superoxide dismutase and copper distribution in stress. *Journal of Dairy Science*. 74:3078.