NISEB JOURNAL Vol. 11, No. 1 , January 31, 2011
Printed in Nigeria

1595-6938/2011 $12.00 + 0.00

http://www.nisebj.org

NISEB 2010039/11202

Anti-inflammatory medicinal plants: The effect of three species on serum antioxidant system and lipids levels in rats

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(Received August 25, 2010; Accepted November 10, 2010)

ABSTRACT: **Aim of Study:** To determine the effect of ethanolic leaves extracts of three medicinal plants; *Globumetulla branuii, Acalypha torta* and *Lecaniodiscus cupanioides* on the serum antioxidant system and lipid profiles of albino rats. **Materials and methods:** The pulverized leaves of the plants were each extracted in cold 80 % ethanol and the extracts were administered orally to different groups of test rats at a dose of 200 mg kg^{-1} for 21 days. **Results:** The extracts exhibited significantly lower ($p<0.05$) superoxide dismutase and catalase activities in the serum of treated rats when compared to the control and vitamin E treated rats. The MDA level which was used as an index of lipid peroxidation was significantly higher ($p<0.05$) in rats treated with *L. cupanioides* extract (4.20 ± 0.35 mMol L^{-1}) compared to the control rats (2.87 ± 0.23 mMol L^{-1}). Evaluation of lipid profile showed that triacylglycerides levels were observed to be significantly ($p<0.05$) lower in all the extract treated rats in the following order, *L. cupanioides* > *A. torta* > *G. branuii*. Extracts of *G. branuii* and *L. cupanioides* also exhibited significant ($p<0.05$) reduction of total cholesterol. In addition, *G. branuii* cleared LDL completely and elevated HDL. The lipid lowering abilities of the extracts were better than that of vitamin E except *G. branuii*. **Conclusions:** The study suggests that the mode of anti-inflammatory action of these plants extract might be due to the modulation of oxidative status in cells as well as the regulation of lipid levels of the treated rats. The presented data lends support to the use of these plants in ethno-medicine practice in rural Nigerian communities.

Key Words: Inflammatory diseases, medicinal plants, oxidative status, lipid profile.

Introduction

Cells response to tissue injury caused by infections, irritation, excessive reactive oxygen and nitrogen species (RONS) by becoming inflamed. Inflammation due to the action of RONS is on the increase in recent times, due mainly to environmental pollution, stressful lifestyles, change in diet, and drugs abuse such as smoking and alcohol consumption (Dongmei *et al*., 2006; Pala and Tabakcioglu, 2007).

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Studies have reported a critical involvement of RONS in pathogenesis of many diseases involving inflammation such as rheumatic pains, arthritis, ischemia/reperfusion injury, atherosclerosis and Alzheimer's (Choi and Hwang, 2005, Ceriello and Motz, 2004). Subsequent on these reports, for years, the intuitive assumption has been that all RONS are bad for the cell. The consequent response has been to try to suppress RONS, hoping to prevent or even reverse RONS-related biological damage. However, it is the conditions that increase production of RONS which are the culprit in the increasing occurrence of inflammatory related diseases (Rattam, 2006).

African traditional systems of health care have undergone a major revival in the last thirty years (Meissner, 2009). Several factors are encouraging this revival. Factors such as the non-existence of effective conventional synthetic drug treatments for many chronic diseases such as asthma, arthritis, hypertension, diabetes; cost and the toxic side effects of conventional medicine have made people turn to these ethno-medical methods of treatments. For example, research have established that non-steroidal anti-inflammatory drugs (NSAID) has adverse effects on the gastrointestinal tracts, kidneys and elevates blood pressure (Horl, 2010).

Research into medicinal plants therefore has been intensified in recent times, with the real hope of finding alternatives to synthetic drugs for the management of the chronic diseases. Studies have shown a high correlation between pharmacological activity/clinical use of plant isolates and their established use as herbal medicine (Iwu, 1993; Schuster *et al.,* 1999). However, there is a dearth in scientific findings about the mode of pharmacological activities of medicinal plants used by traditional medicine practitioners.

For this study, leaves of three plants *Globumentulla branuii Engl van Tiegh* (Loranthaceae), *Acalypha torta* Muell (Euphorbiaceae) (Croton flower) and *Lecaniodiscus cupanioides Planch* (Sapindaceae) used in folkloric medicine for treatment of inflammatory diseases were selected from ethno-medical survey; based on the frequency of usage in decoction prepared for the treatment of inflammatory conditions such as arthritis and rheumatic pains.

This study therefore is aimed at evaluating the effect of the three medicinal plants extracts on the indices of oxidative status of cells and the lipid parameters *in vivo* that could highlight the most probable mode of the anti-inflammatory activities of the plants. Our approach is predicated on the role of reactive oxygen species and lipids in precipitating inflammatory conditions.

Materials and Methods

Plants collection and identification

The leaves of *G. branuii* and *L. cupanioides* were collected by Mrs. Fagbemi, a herbal medical practitioner in Mushin market, Lagos, Nigeria, at various times between January – February 2007. The plants were authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan. Voucher specimens (*G. branuii*: FHI: 10774 and *L. cupanioides*: FHI: 10775 were prepared and deposited at the herbarium of the Institute. The leaves of *A. torta* were collected by Dr. C. N. Ezekwesili in Abagana, Anambra state. It was also identified and authenticated at the Department of Botany, University of Nigeria, Nsukka, by a taxonomist Mr. E.C. Ekekwe. Voucher specimen No. 8256 were prepared and deposited at the herbarium of the International Centre for Ethnomedicine and Drug Development (INTERCED), Nsukka.

Preparation of Plant extracts

The leaves of the plants were air dried under shade for 14 days and pulverized. The pulverized leaves were extracted cold by allowing the plants materials to be covered in the solvent for 24 h on the bench. *G. branuii and L. cupanioides* (200 g) was extracted with 1.5 L of 80 % ethanol, while 100 g of *A. torta* was extracted in 1 L of ethanol of the same strength. The extract solutions were filtered through Whatman No. 4 filter paper and the filtrate was concentrated in a water bath (Philips Harris Ltd, England) at 40 ˚C for 8 h. The extracts were stored at 4 ˚C until used. The extracts were weighed and the percent yield calculated by the formula:

$$\frac{\text{Weight of extract}}{\text{Initial weight of plant material}} \times 100$$

Test animals

Thirty-six normal albino rats used for the experiment were purchased from the rat colony at Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. The animals used in this study followed the requirement and guideline on care and handling of animals in animal experimentation (Hideaki, 2007). The number of animals used was kept to minimum necessary for meaningful interpretation of data. The animals were housed in standard rat cages to minimize their discomfort and were fed with commercial livestock feed (Topfeeds Nigeria Ltd) and water *ad libitum*.

Animal treatment

The rats weighing between 180-220 g were sorted based on their weights and grouped into 6 with 6 rats each. The groups were 1– Control (Untreated), 2 – Vitamin E (reference antioxidant), 3 - *G. branuii*, 4 - *L. cupanioides* 5 - *A. torta* treated groups. The extracts solutions of 1 mL/200mg kg^{-1} were administered orally to the test groups for 21 days. The control group received 1mL of 5 % Tween 20 only. The animals were fasted overnight on day 22, sacrificed on day 23 and blood collected. The blood was centrifuged at 1500 × *g* for 5 min and serum was separated for enzymes assay.

Enzyme assays

Chemicals

Hydrogen peroxide (H_2O_2), Epinephrine ($C_9H_{13}O_3N$), Tween 20 and α-tocopherol (vitamin E) were purchased from Sigma-Aldrich, Germany. All other chemicals used, including solvents, were of analytical grade.

Determination of Superoxide dismutase (SOD) activity

The method of Sun and Zigma, (1978) was adopted. The reaction mixture (3 ml) contained 2.95 ml sodium carbonate buffer (0.05 mM, pH 10.2) and 0.02 ml of serum, 0.03 ml epinephrine (3 M) in 0.005 N HCl was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.02 ml of water and 0.03 ml of substrate (epinephrine). Enzyme activity was determined by measuring the change in absorbance at 480 nm for 3-5 min. An extinction coefficient of 4020 $M^{-1}cm^{-1}$ was used to calculate enzyme activity and expressed in Unit mg $protein^{-1}$.

Determination of Catalase (CAT) activity

Serum catalase activity was determined according to the method of Beers and Sizer as described by Usoh *et al.*, (2005) by measuring the decrease in absorbance at 240nm due to the decomposition of H_2O_2 in a UV recording spectrophotomer (Ultraspec 3100pro, Amerchan Biosciences, UK) at intervals of 60 s for 5 min. The reaction mixture (3 mL) contained 0.1 mL of serum and 2.9 mL of 30 mM H_2O_2 in phosphate buffer (50 mM, pH 7.0). The test readings were read against blank devoid of serum. An extinction coefficient for H_2O_2 at 240 nm of 40.0 $M^{-1}cm^{-1}$ (Aebi, 1984) was used for the enzyme activity calculation and Unit mg $protein^{-1}$.

Determination of lipid peroxidation index

The formation of TBARS (thiobarbituric acid reactive substances) was used as an index of lipid peroxidation according to the method of Niehaus and Samuelson as described by Usoh *et al*., (2005).

Triacyglycerides and cholesterol level

The concentrations of triacyglycerides (TAG), total cholesterol (TC) and high density lipoprotein (HDL-cholesterol) in plasma were determined enzymatically using commercially available test kits

(Boehringer, Mannheim, Germany). Low density lipoprotein (LDL-cholesterol) was calculated by the Friedewald formula:
LDL-C = TC – (HDL-C + TAG). Very low density lipoprotein (VLDL) was calculated by Tietz, (1986) formula: VLDL = TG/5

Statistical analysis

The data were subjected to one-way analysis of variance and difference between the means was determined by Duncan's multiple range tests ($p \leq 0.05$). Results are expressed as MEAN ± SD (Standard Error of Mean). The analysis was performed using Statistical Package for the Social Science15.0 for windows (SPSS 15.0).

Results

Plant extract yield

The ethanol plant extracts yields were 5.19 % (*G. branuii*), 8.66 % (*L. cupanioides*) and 20.15 % (*A. torta*).

Superoxide dismutase (SOD) *activity*

SOD activity was significantly lowered ($p<0.05$) in the serum of *G. branui, A. torta and L. cupanioides* extracts'-treated rats compared to control and vitamin E (Fig. 1). *A. torta* extract-treated rats showed lower SOD activity compared to *G. branui* and *L. cupanioides* treated but this was not significantly different ($p<0.05$). Furthermore, there was significant increase ($p<0.05$) in activity of vitamin E treated rats compared to control. Nevertheless, there was no significant difference ($p<0.05$) in SOD activity between control and vitamin E.

Catalase (CAT) *activity*

Similarly, catalase activity was significantly lowered ($p<0.05$) in the serum of all the extracts'-treated rats compared to control and vitamin E. However, amongst the extracts'-treated rats, *A. torta*-treated had significant ($p<0.05$) higher catalase activity than *G. branui,* and *L. cupanioides* treated rats (Fig. 2).

Lipid peroxidation

L. cupanioides showed a marked increase ($p<0.05$) in lipid peroxidation product (MDA) compared to both control and vitamin E. There was no difference ($p<0.05$) between *G. branuii,* and *A. torta* extracts-treated animals.

Lipid profile

The plants extract affected the plasma lipid profile of the treated rats at varying degrees (Table 1). TAG was lowered in the order of *L. cupanioides* > *A. torta* > *G. branuii*. Likewise, *G. branuii* and *L. cupanioides* extracts exhibited cholesterol lowering ability, which was not observed in the control. In addition, *G. branuii* increased the proportions of HDL in the plasma of extract-treated rats showing the same property as vitamin E, whereas *L. cupanioides* and *A. torta* caused a lowered HDL compared to *G. branuii*. Furthermore, *G. branuii*'s extract cleared LDL from plasma of treated rats equally as in vit E administered. Similarly, the extracts lowered VLDL than control, this was in the order *L. cupanioides* > *A. torta* > *G. branuii*.

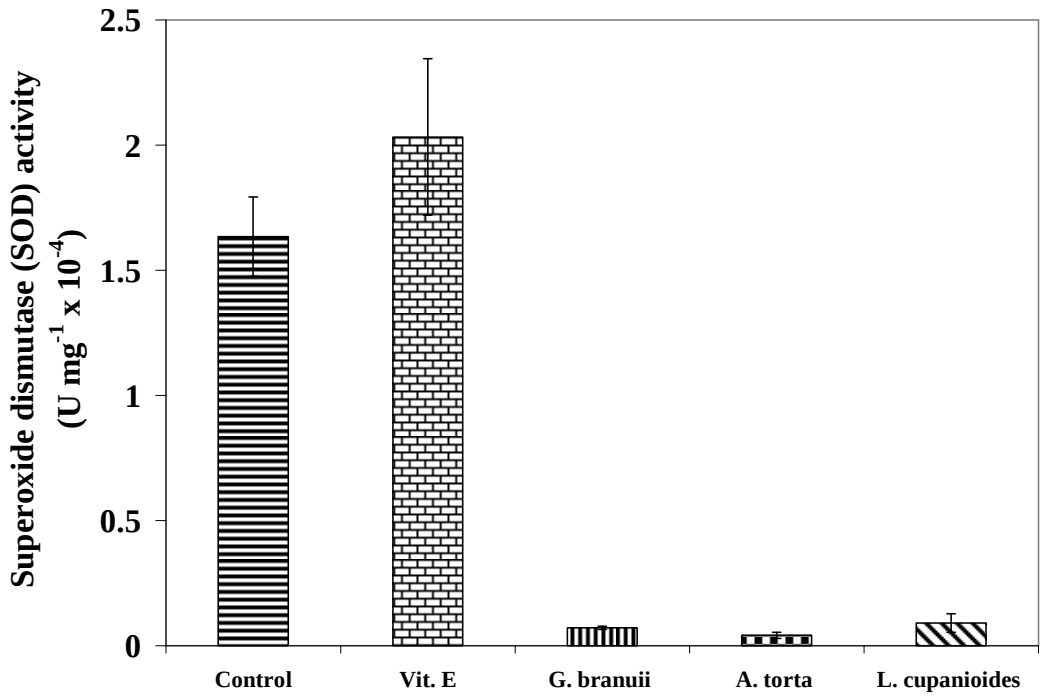

Fig. 1: Superoxide dismutase activity (U mg^{-1} $x10^{-4}$) in control, vit E and extracts-treated rats

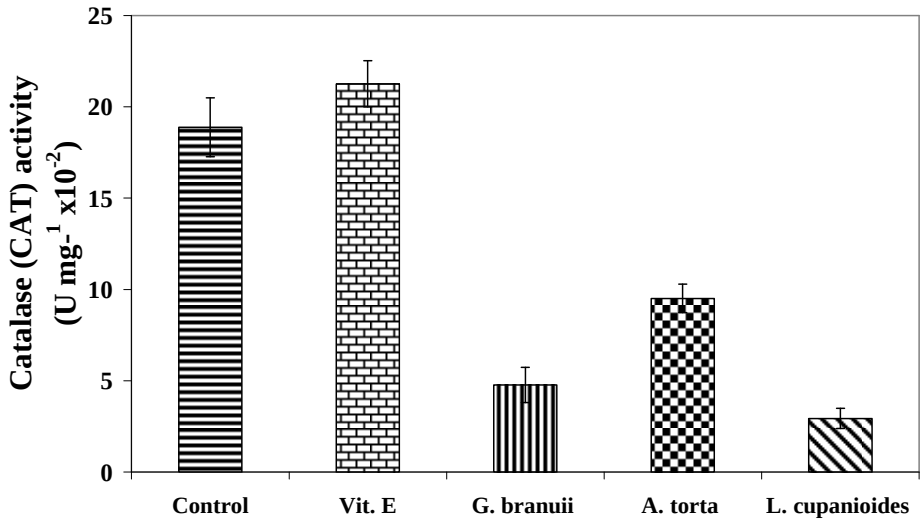

Fig. 2: Catalase activity in (U $x10^{-2}$) in control, vit E and extracts-treated rats

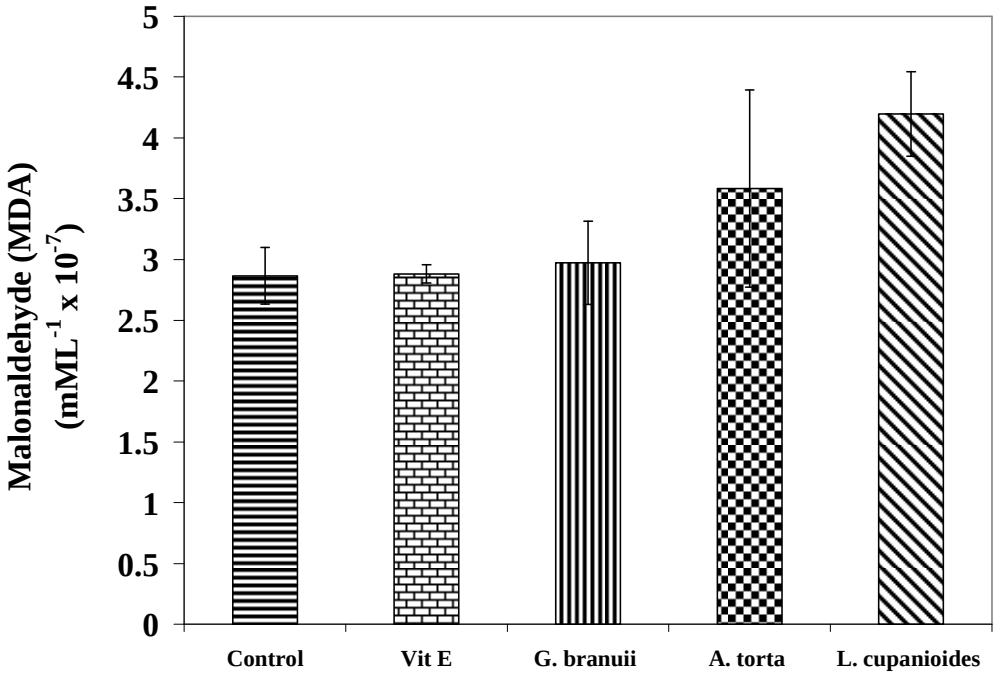

Fig. 3: Malonylaldehyde (MDA) in control, vit E and extracts-treated rats

Table 1: Lipid Profile of Control, Vitamin E and Extracts'-Treated Rats (mMol L^{-1} x 10^{-1})

Lipids	**TAG**	**TC**	**HDL**	**LDL**	**VLDL**
Ext/Ref. Antiox.					
Control	145.2±9.7	71.2±4.03	43.8±3.8	4.8±0.97	29.2±2.2
Vitamin E	124±6.4*	4.8±0.97	65.4±2.3*	0*	24±1.3*
G. branuii	128.4±5.0*	47.8±6.06*	71±4.5*	0*	25.8±1.06*
L.cupanioides	95.8±4.8*	52.01±8.6*	34.6±3.7*	13±2.09*	19.2±0.86*
A.torta	113.8±2.9*	72.2±3.88	35.8±2.18*	12.4±5.95*	22.6±0.4*

Values are ± SEM
*Significant at $p \leq 0.05$
Ext/Antiox— Extracts/Reference Antioxidant

Discussion

This work was designed to examine the effect on antioxidant enzymes and lipids profile on administration of ethanolic leaves extracts of three medicinal plants (*G. branuii, A. torta and L. cupanioides*) claimed by traditional medicine practitioners to be effective for the treatment of inflammatory conditions. The extensive use of the three plants in herbal remedy for treatment of inflammatory conditions makes it important that studies to determine their effect on parameters which are implicated in pathogenesis of these conditions particularly arthritis, atherosclerosis and cardiovascular diseases be investigated.

The inhibitory effect of the plants extracts on two major enzymatic antioxidants (SOD and CAT) compared to the control and vitamin E, suggests the creation of oxidative stress in the treated rats. This inhibition might be due to the plants extracts possessing pro-oxidant properties. Studies on many medicinal plants have demonstrated the crucial role of oxidative stress as pathophysiological mechanism in diseases or experimental models (Odukoya, 2010). Secondary metabolites from plants have also been showed to achieve their anti-inflammatory activity through their pro-oxidant/antioxidant mechanism (Sandur *et al*., 2007). Furthermore, major plant metabolites known for their anti-inflammatory activities are plant phenols such as flavonoids (Gonzallez-Gollago *et al*., 2007); and it has been reported that the ability of these plant phenols to form the phenoxyl radicals and the stability of these derived radicals are important in anti-inflammatory activities of phenols (Ruiz *et al*., 1996). This is corroborated by our earlier studies on *G. branuii*'s ethyl acetate fraction which contain a phenol naringin (Okpuzor *et al*., 2009), found in citrus fruits such as grape and grapefruit. Naringin has been reported to have strong anti-inflammatory activity, mediated through its pro-oxidant mechanism (Buhler and Miranda, 2000; Pereira *et al*., 2007).
However, an elevated serum level of MDA observed in *L. cupanioides*-treated rats suggests that it might have created oxidative stress *in vivo*.

The lipid lowering effect of all the extracts further point to their most probable mode of action, particularly when compared to the mode of action of conventional non-steroidal anti-inflammatory drugs which is by inhibition of prostaglandin synthesis (Graham *et al*., 2001). Synthesis of prostaglandin which mediates inflammation, pain, and fever is catalyzed by COX-2 from the substrate arachidonic acid – a fatty acid (Moreno *et al*., 2001). Therefore, the lowering of the lipids concentration by the extracts indicates slowing down the pathway to the synthesis of prostaglandins, thereby arresting inflammation.

The effect of the extracts of *G. branuii* and *L. cupanioides* on total cholesterol (TC) is remarkable. Lipids, particularly cholesterol, have been implicated in the pathogenesis of inflammatory diseases, such as rheumatism, arthritis, and cardiovascular diseases (Darlinghton and Stone, 2001). This observation is quite significant as clearance of cholesterol from serum suggests lower risk of development of chronic inflammatory related diseases. Furthermore, the significant increase in high density lipoproprotein cholesterol (HDL-C) indicates low artherogenic index (calculated from the ratio of TC and HDL-C which

is less than 1) in *G. branuii* extract-treated rats and also signifies a reduced risk of developing inflammatory diseases. Experimental evidences suggest that this can have a major vascular protective effect in humans especially in atherosclerotic-vascular disease and hypertension (Shah *et al*., 2001). This protective effect of HDL may be due in part to its ability to inhibit the oxidative modification of LDL (Navab *et al*., 1991). In addition, studies have also shown that HDL-C attenuates the cytokine-induced expression of adhesion molecules (VCAM-1, E-selectin), which mediates fatty streak formation (Choi and Hwang, 2005).

Moreover, the complete clearance of LDL from *G. branuii*-treated rats' plasma and the significant lowering of VLDL in all the extracts'-treated rats are important observations. Researchers have shown that oxidation of LDL-cholesterol could lead to induction of adhesion and influx of monocytes; an increased uptake of cholesterol by macrophages which is one of the events leading the formation of fatty streak in artherosclerotic lesions in arterial walls (Bowry *et al*., 1992, Singh *et al*., 2005). In conclusion, the data from this study lends support to the use of these plants in the management and/or control of chronic diseases due to inflammation particularly arthritis, hypertension and cardiovascular diseases (CVD).

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