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Investigation on Protective Effect of Tapinanthus Globiferus Leaf Extract against Pb^{2+} Induced Hypertension and Ulcer

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Abstract: ***Hypertension and ulcerogenic diseases have been reported to have very high recurrence and mortality rate especially in complicated cases. The present investigation on this study is to assess the antioxidant and anti-ulcerogenic potentials of Tapinanthus globiferus leaf extract against Pb^{2+} -induced hypertension and ulcerogenic diseases. Phenolic content of different solvent extract of the plant sample was evaluated while mucosal epithelial tissue homogenate excised from wistar rat stomach was used for determining gastroprotective potential of Tapinanthus globiferus leaf extract. Metal chelation ability and nitric oxide radicals scavenging ability of the leaf extract were also assessed as antioxidant indices for anti-hypertensive poential of the extract in vitro.***

The results revealed significant ($P<0.05$) anti-ulcerogenic potential of the extract where (79.70%) highest percentage inhibition of ulcer index was obtained at highest (160mg/ml) extract concentration. Besides, the antioxidant potential demonstrated against nitric oxide and metal chelation was highly significant ($P<0.05$).

The extract demonstrated higher percentage inhibition (96.86%) of metal chelation and (78.18%) was also observed against nitric oxide radicals at highest extract concentration (160mg/ml).

The high potent protective effect exhibited by Tapinanthus globiferus leaf extract may be attributed to its inherent antioxidant phytochemicals. Hence, Tapinanthus globiferus leaves could possibly offer protection against heavy metal-induced hypertension and ulcerogenic diseases.

Keywords: Tapinanthus globiferus, anti-ulcerogenic, hypertension, nitric oxide, antioxidant.

I. INTRODUCTION

Lead has been reported to cause endothelial and epithelial injuries while epidemiological investigations have revealed a link between lead exposure and subsequent development hypertension (Schafer et al., 2003). Research findings have equally demonstrated that lead exposure causes hypertension and cardiovascular diseases by promoting oxidative stress, limiting nitric oxide availabilty and signaling(Rimbach et al., 1999). Free radicals and other reactive oxygen species (ROS) are by-products of cellular metabolism and cells normally have a number of mechanisms to defend against damage induced by free radicals (Halliwell, 1994). Oxidative stress occurs whenever the production of ROS exceeds the ability of cells to readily detoxify the reactive intermediates or easily repair the resulting damage (Rimbach et al., 1999).

All forms of life usually maintain a reducing environment within their cells by enzymes through a constant input of metabolic energy. Imbalances in this normal redox state could lead to wanton production of peroxides and free radicals that damage macromolecules of the cells such as lipids, proteins and DNA (Amarowics et al., 2004). Besides, myriad of studies on phytomedicines have reported that phenolic compounds protect against oxidative stress (Miron et al., 2005). Some of these medicinal plants have been investigated for their antioxidative properties and used for treatment of various diseases (Weisburger, 1999). Most of the bioactive metabolites from these plants especially flavonoids demonstrated potent antioxidant activity in vitro and in vivo (Usoh et al., 2005).

Many synthetic antioxidants and metal chelator components have also exhibited toxic or mutagenic effect coupled with suppression of body immunity which have shifted attention towards naturally occuring antioxidants (Fejes et al., 2000). Tapinanthus globiferus is grown specifically for its essential oils in its leaves and stems where thymol, eugenol, citral, geraniol and linalool have been extracted (Sulistiarini et al., 1999).

This study is aimed at investigating the protective potentials of Tapinanthus globiferus leaf extract against Pb^{2+} -induced hypertension and ulcerogenic diseases.

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II. MATERIALS AND METHODS

A. *Collection of Plant Sample*

Fresh leaves of Tapinanthus globiferus were fetched from a reserved virgin forest neear Ikere-Ekiti township, Ekiti State, Nigeria. The plant was authenticated at the herbarium centre of Department of Agricultural Technology, Federal Polytechnic, ado-Ekiti.

B. *Preparation of Plant Extracts*

The aqueous extract was prepared by extracting 150g of powdered sample in cold sterile distilled water, aggitated with mechanical shaker, and filtered via buchner funnel with No 1 whatman's filter paper, frozen at -40^{0}C and dried with freeze dryer for 72hrs and percentage yield of 11.33% was obtained (Berln and Schaller,2008). 120g each of powdered samples was extracted with solvent combination (70%) ethanol and ethyl acetate respectively. The mixture was decanted and filtered with No 1 Whatman's filter paper which measured up to 600mls and evaporated to dryness to give 9.96% yield.

C. *Determination of Total Phenolics*

The total phenolic content of the T.globiferus extracts was determined with (Singleton et al., 1999) method. A reaction mixture of (v/v) Folin-Ciocateu reagent and (w/v) sodium carbonate was added to the extracts and the mixture was vortexed and incubated at 40^{0}C for 30min after which the absorbance was measured at 765nm.

D. *Determination of Metal Chelation Ability*

The Pb^{2+} chelating ability of the extracts was determined using (Minotti and Aust, 1987) method. Freshly prepared $PbSO_4$ (150µL) was added to mixture containing 168µL of 0.1molL^{-1} Tris-HCl (7.4), 218µL saline and (0-25µL) extracts, incubated with addition of 13µL, 0.25% 1,10-phenanthroline (w/v) and absorbance measured at 510nm.

E. *Determination of Nitric Oxide [NO*] Scavenging Ability*

Nitric oxide scavenging ability of the extracts was determined by (Jagetia and Baliga, 2004) method. 10mM sodium nitroprusside prepared in 0.5mM phosphate saline buffer (pH 7.4) was added to the xtracts and vortexed. The mixture was incubated, mixed with Griess reagent and incubated at room temperature for 30min and absorbance was measured at 540nm.

F. *Determination of Ulcerogenic Index [In vitro]*

This was determined using (Mabrouk et al., 2009) modified method. Rats were decapitated and the stomach mucosal epithelial layer curvatures were excised, placed on ice and weighed. The tissue was subsequently homogenized in cold saline (1/10 w/v) with about 10 up and down strokes at approximately 1,200 rpm in a Teflon glass homogenizer. The homogenate was centrifuged for 10min at (3,000 x g) to yield a low speed supernatant which was used for the determination of percentage inhibition of ulcer index calculated as:

$$\% \text{ Inhibition} = \frac{[\text{Lesion gastric acid index} - \text{Lesion gastric acid in test}] \times 100}{\text{Lesion gastric acid in control}}$$

III. RESULTS

Table 1: Polyphenolic contents of different solvent extracts of Tapinanthus globiferus

Extract	Phenols(mg/TE/g)	Flavonoids (mg/QE/g)	Flavonols (mgQE/g)
Aqueous	240.16±0.17	32.43±0.84	23.55±0.04
Ethanol	94.01±2.24	38.69±1.20	15.64±1.55
Ethyl acetate	57.76±4.30	22.28±0.52	2.57±0.10

Data expressed as mean±SD (n=3), TE and QE are tannic acid and quercetine equivalent

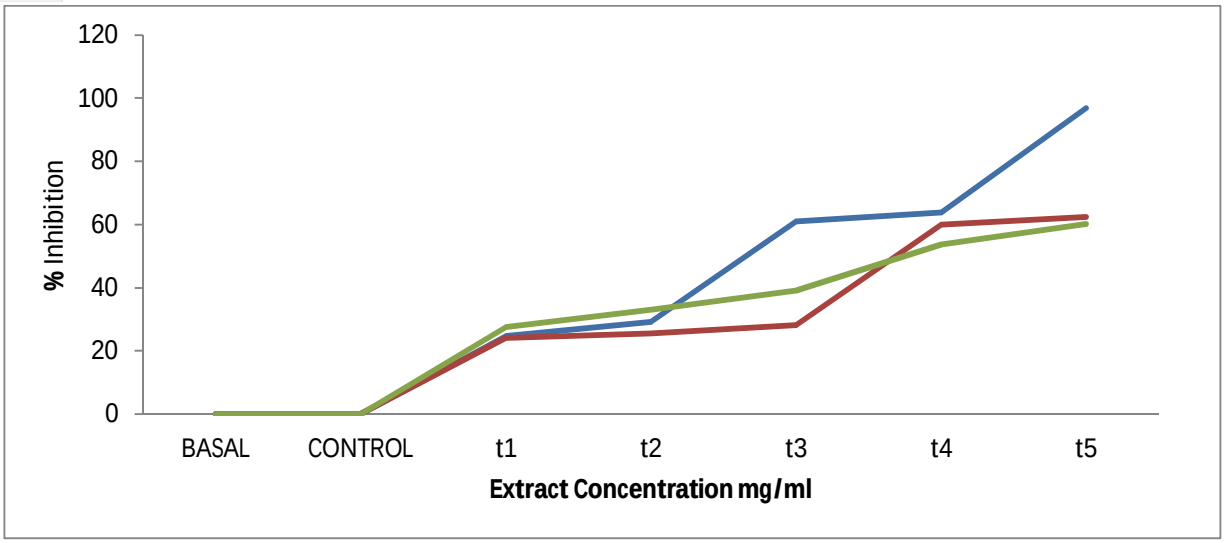

Fig. 1: Pb^{2+} - Chelating ability of Tapinanthus globiferus leaf extracts

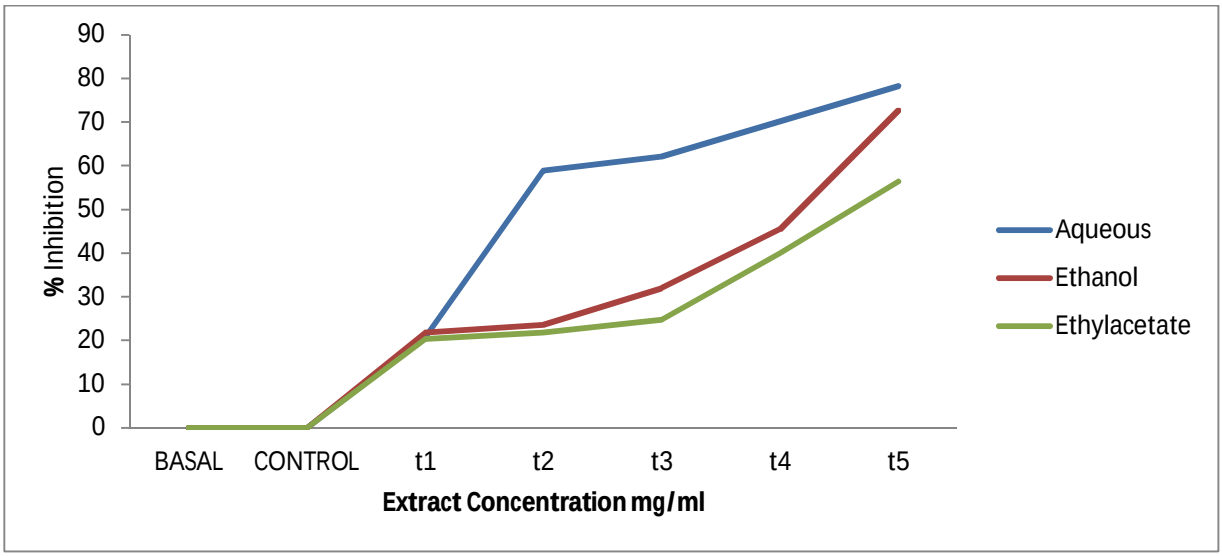

Fig. 2: Nitric oxide radicals scavenging ability of Tapinanthus globiferus leaf extracts

Table 2: Inhibitory effect of T. globiferus crude extract against Pb^{2+} induced ulcer in rats

Extract (mg/ml)	Lesion gastric acid absorbance	% Inhibition	IC_{50} (mg/ml)
Basal	1.78	-	2.41±0.02
Control	3.45	-	Y= -7.27 ln (x) + 82.88
t_1 (10)	3.33	70.70	
t_2 (20)	2.67	72.44	
t_3 (40)	1.33	71.12	
t_4 (80)	0.66	72.61	
t_5 (160)	0.33	79.70	

*Results are expressed as means of three experiments in duplicate ± standard deviation

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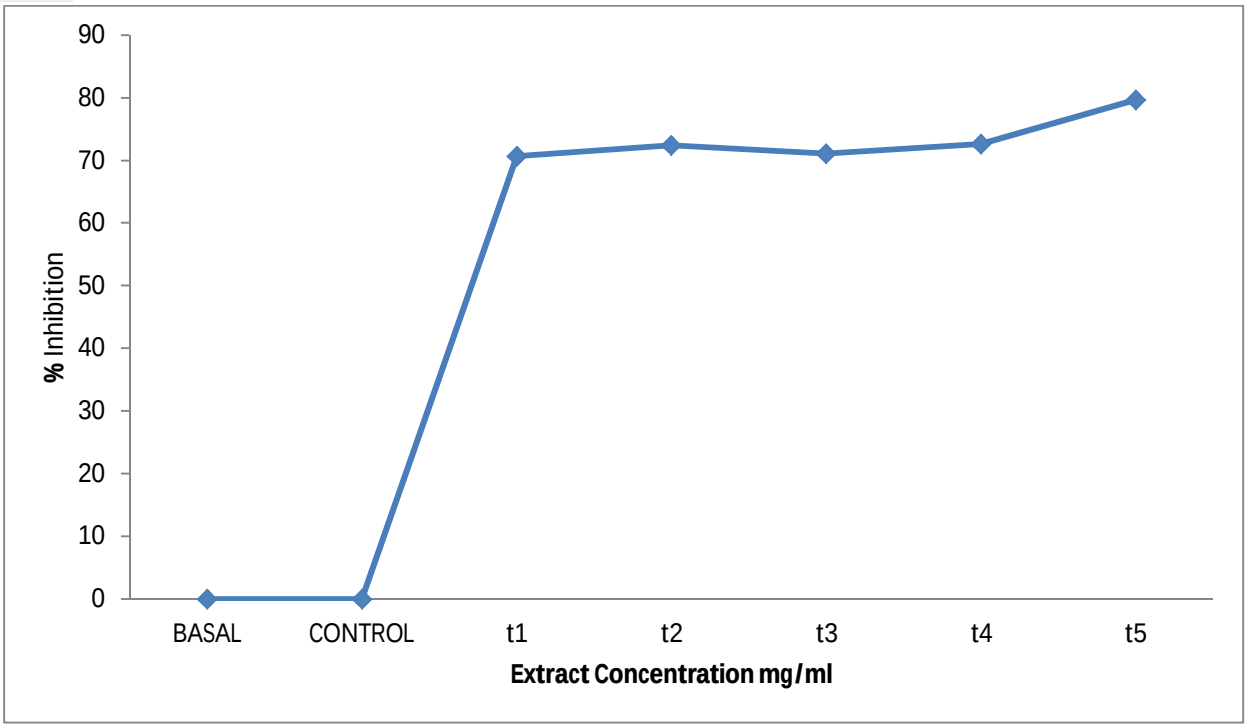

Fig. 3: Inhibitory effect of Tapinanthus globiferus crude extract against Pb^{2+} induced ulcer in rats

IV. DISCUSSION

The results obtained in this study reveal higest phenolic contents (240.16mgTE/g) total phenols, (32.43mgQE/g) total flavonoids and (23.55mgQE/g) total flavonols in Tapinanthus globiferus aqueous extract while ethyl acetate extract had the lowest concentrations (57.76mgTE/g) total phenols, (22.28mgQE/g) total flavonoids and (2.57mgQE/g) total flavonols respectively. High phenolic contents of plants has been reported to contribute directly to antioxidative action and development of reducing power of such plants (Duh et al., 1999). The results show that aqueous extract of Tapinanthus globiferus has higher reducing and antioxidant potentials than ethanolic and ethyl acetate extracts and this might be due to its higher hydrogen donating ability (Shimada et al., 1992). Besides, chelating agents usually inhibit radical mediated oxidative chain reactions in biological system and consequently improve human health and food safety. Tapinanthus globiferus extract demonstrated high significant ($P<0.05$) inhibitory potential against lead and nitric oxide radicals in which highest percentage inhibition (96.86%) of extract metal chelating ability and (78.18%) nitric oxide scavenging ability of the extract respectively were obtained at (160mg/ml) highest extract concentration as shown in Fig. 1 and 2. This further suggests the extract as an anti-hypertensive agent because it could easily chelate lead and prevent the body inherent neurotransmitter (NO) from combining with oxygen to form nitrite ion (NOO^{-}) which is a reactive oxygen species which can easily inhibit the release of neurotransmitter that is usually released in circulation in order to activate guanylate cyclase and cGMP in vascular smooth muscles where protein kinase G activates the phosphatases that inactivate myosin light chain muscles which are directly involved in muscle contraction that brings about high blood pressure leading to hypertension. The findings that heavy metals such as mercury and lead cause significant increase in MDA and thiobarbituric acid content in some internalized tissues like liver, brain and kidney and further induce lipid peroxidation (Oboh et al., 2007) which is an index of oxidative stress that damage the tissues giving rise to myriads of diseases such as cancer, arhritis, alzheimer, schizophrenia, and coronary heart disease. The result from Table 2 and Fig. 3 is in agreement with this assertion where significant ($P<0.05$) level of gastric acid from lesions on the mucosal epithelial layer of the excised stomach homogenate was indicated. However, the high inhibitory potential of the extract suggest that Pb^{2+} chelation may be one of the possible mechanisms through which antioxidant phytochemicals from Tapinanthus globiferus prevent ulcer in the stomach mucosal layer by forming complex with Pb, thus preventing initiation of lipid peroxidation in the mucosal layer which normally result into ulcerated mucosal lesions and increased acidity of gastric acid mixture (mucosal hydrochloric acid) that mop up the mucus and this could possibly give rise to either (Aguawa and Mittal) gastric or peptic-ulcer. The results of this study suggest that phenolic content and antioxidant activity of the Tapinanthus globiferus extract could offer protection against Pb^{2+} -induced hypertension and ulcerogenic diseases.

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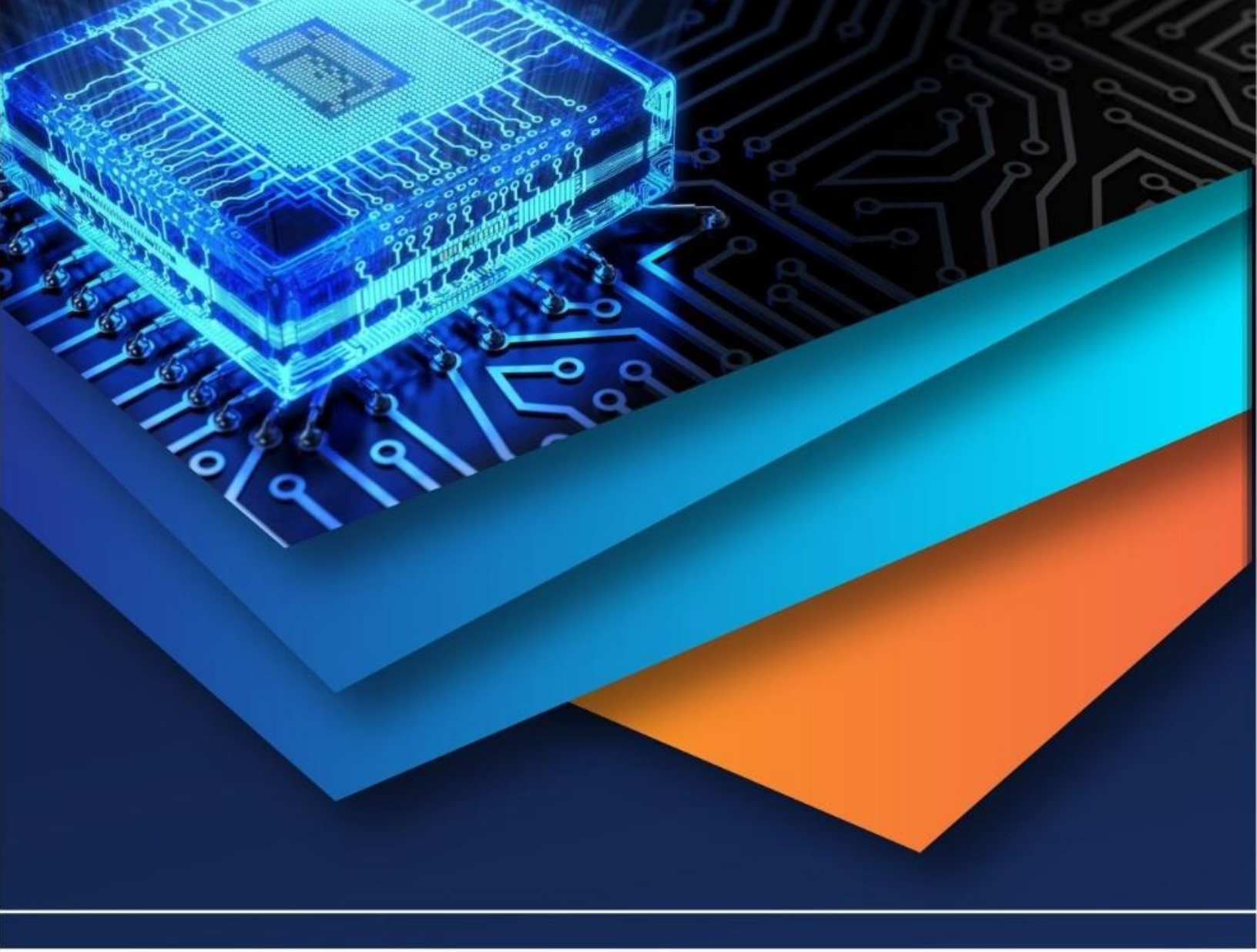

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