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In Vitro Effect of n-Hexane Extract of *Gongronema latifolium* Leaves on Oxidants'-Induced Assault and Activities of delta Amino levulinic Acid Dehydratase and purinergic Enzymes in Hepatic and Cerebral Tissues of Wistar Rats

Ale, Ebenezer Morayo[1*]; Yakubu, Ojochenemi Ejeh[1], Asuelimen, Steve Osagie[1]; Shadrach, Philip[1]; Olorundare, Bunmi Olaoluwa[2]; Asaph, Peace Magaji[3]

[1]Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari, Taraba State, 670101, Nigeria.
[2]Cocoa Research Institute of Nigeria, Ibadan, Oyo State, 200211, Nigeria.
[3]Department of Chemical Sciences, Faculty of Pure and Applied Sciences, Federal University Wukari, Taraba State, 670101, Nigeria.

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ABSTRACT

Gongronema latifolium is a medicinal plant with numerous benefits. Several studies have linked these medicinal actions to the presence of some bioactive compounds in the plant. The present study sought to investigate the effect of n-hexane extracts of leaves of *G. latifolium* on sodium nitroprusside (SNP) and quinolinic acid (QA)-induced lipid peroxidation as well as its modulatory effect on the activities of delta aminolevulinic acid dehydratase (δ- ALAD) and purinergic enzymes [5'-nucleotidase and nucleoside triphosphate dihydrolase (NTPDase)]. The results showed that the extracts markedly (P< 0.05) inhibited both SNP and QA-induced lipid peroxidation in a concentration dependent manner. In addition, the extracts caused a non-significant (P< 0.05) increase in the activity of δ-ALAD when compared with the control. Finally, the results of purinergic enzymes' assays revealed that the extract exhibited significant (P< 0.05) inhibition of NTPDase and 5'-nucleotidase activities in the rat tissues with increasing concentration. In any case, it is rational to suggest that *G. latifolium* could be an ideal intervention in the management of radical related degenerative diseases and in search of a promising novel purinergic targeting drugs.

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*Corresponding author: e- mail: ebenezerale@gmail.com or alebenezer@fuwukari.edu.ng

INTRODUCTION

Lipid peroxidation is a crucial step in the pathogenesis of several diseases in adult and infants. Reactive oxygen species (ROS) are generated spontaneously in cells during metabolism and are implicated in the aetiology of different degenerative diseases, such as heart diseases, stroke rheumatoid arthritis, diabetes and cancer (Oloyede & Afolabi, 2012). These have however been terminated by antioxidants by the removal of free

radicals intermediates and the inhibition of the other oxidative reactions. Considering the fact that the body's internal production of antioxidants is not enough to neutralize all the free radical, there is need to supplement it with constant intake of dietary antioxidants in the quest to maintain a healthy state (Oloyede and Afolabi, 2012). Consequently, there is a great deal of interest in edible plants that contain antioxidants and health promoting bioactive and therapeutic agents. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most active of these bioactive compounds (phytochemicals) are alkaloids, flavonoids, tannins and phenolic compounds (Chhetri, 2008). *Gongronema latifolium* commonly known in the southeast of Nigeria as Utazi is a climbing herb that has made name as a special grade vegetable, and is widely trusted to have strong nutritional and medicinal values (Enemor *et al.,* 2014). It has a very wide spread distribution as it is easily propagated. *Gongronema latifolium* is utilizable in many different ways in different places to prepare delicacies in homes. Locally, apart from its nutritional flavor, it is reported to possess several medicinal properties including antimalarial, antidiabetic, and antihypertensive activities (Enemor *et al.*, 2014).

There are evidences that purinergic signaling is involved in processes associated with health and disease, including non-communicable, neurological, and degenerative diseases. These diseases strike from children to elderly and are generally characterized by progressive deterioration of cells, eventually leading to tissue or organ degeneration. These pathological conditions can be associated with disturbance in the signaling mediated by nucleotides and nucleosides of adenine, in expression or activity of extracellular ectonucleotidases. For instance, ectonucleotidases such as nucleoside triphosphate diphosphohydrolase (NTPDase) and 5′-nucleotidase play a role inthe regulation of extracellular ATP levels and their activities have been shown to increase in pathophysiological disorders like as atherosclerosis, hypertension, cancer, epilepsy, Alzheimer's disease, Parkinson's disease and multiple sclerosis (Bagatini *et al*., 2018) .However, there is paucity of experimentally based information on the antioxidant *G. latifolium* on some harmful oxidants as well as its modulatory effect on the activities of some key enzymes. Hence, the bases for this study to evaluate the effect of n-hexane extract of *G. latifolium on* sodium nitroprusside and quinolinic acid-induced assault and activities of purinergic enzymes and delta aminolevulinic acid dehydratase in rat cerebral and hepatic tissues.

MATERIALS AND METHODS

Chemicals and Reagents

Adenosine monophosphate (AMP), thiobarbituric (TBA) acid, δ-aminolevulinic acid (ALA) and Tris-HCl were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals which were of analytical grade were obtained from standard commercial suppliers.

Collection and preparation of Plant Material

The leaves of *G. latifolium* were collected from the local suppliers in Owo, Ondo state (Nigeria). The leaves of *G. latifolium* were washed with water and dried under shade at room temperature. The dried leaves were grinded with a mechanical blender and stored in a tightly closed nylon bag. The n-Hexane extract of *G. latifolium* was prepared using Soxhlet extractor. 50.0g of pulverized leaves was put into thimble and boiled in n-hexane on a heating mantle at the temperature of 68^0C. The hexane boiled and washed the extract into the round bottom flask. It was allowed to siphon six (6) times. The n-hexane extract was then concentrated in a rotary evaporator and the n-hexane used for the extraction was recovered. The dried extract was stored in refrigerator for further use.

Animals

Adult male Wistar rats weighing 200 – 250 g were used in the experiments. All rats were supplied food and water ad libitum and were kept on a 12-h light/12-h dark cycle in a room with the temperature regulated to 21 – 25^0 C and humidity at roughly 56%. Animals were treated according to standard guidelines of animal care.

Preparation of tissue homogenate

Animals were anesthetized with mild ether, decapitated and the brains and livers removed. The tissues were rinsed thoroughly with cold 50mM Tris-HCl buffer to ensure that they are free from blood stain and immediately homogenized in cold with 50mM Tris-HCl buffer (1:10 w/v), pH 7.4. The homogenate was centrifuged at 4000 rpm for 10 min and the supernatant was decanted and used for assays.

Thiobarbituric acid reactive substances (TBARS) assay

Lipid peroxidation in the tissue was assayed by quantifying TBARS (thiobarbituric acid reactive substances) using the method according to Ohkawa *et al.* (1979). 100µl of the supernatant was incubated with extract (of concentrations between 0-20 mg/ml) for 1hour at 37^0C in the presence of pro-oxidants [10 µMquinolinic acid (QA) or 20 µM sodium nitroprusside (SNP)]. The reaction was carried out in 50mM Tris-HCl buffer (pH 7.4). 200µl of 8.1% SDS (Sodium Dodecyl Sulphate), 500µl of acetate buffer pH 3.4, and 500µl of 0.8%TBA were added to develop the colour reaction. The reaction system was then incubated for 30 minutes

at 100^0C and TBARS was read at 532 nm in UV-visible spectrophotometer.

δ-Aminolevulinic Acid Dehydratase Assay

Cerebral δ-ALA-D activities was assayed according to the method of Sassa (1982) by measuring the rate of formation of the product porphobilinogen (PBG), except that 84mM potassium phosphate buffer, pH 6.4 and 2.4mM ALA were used. Incubations were carried out for 2hrs at 37^0C. The purple reaction products were determined using modified Ehrlich's reagent at 555nm, with a molar absorption coefficient of 6.1 x 10^4 M^{-1} for the Ehrlich-PBG salt.

Purinergic Enzymes

5^1-Nucleotidase Assay

5^1-Nucleotidase activity was determined in a reaction medium essentially as described by Heymann *et al.* (1984) with 80-120µg of protein added. The reaction was mixed and pre-incubated for 10min at 37^0C and initiated by the addition of AMP to a final concentration of 20mM and incubated for 20min.

NTPDase Assay

NTPDase-like activity was determined in a reaction medium as described by *Battastini et al.* (1991). 80-120µg of protein of the enzyme preparation was added to the reaction mixture and pre-incubated for 10min at 37^0C. The reaction was initiated by the addition of ATP to a final concentration of 3.0mM.

For both NTPDase and 5′-nucleotidaseactivities determination, the assays were stopped by the addition of 250 µ L of 10% TCA to a final concentration of 5%. Released inorganic phosphate was measured by the method of Fiske and Subbarow (1925). All samples were run in duplicate or triplicate. Enzyme-specific activities are reported as nano moles Pi released per minute per milligram of protein.

Statistical analysis

The results were expressed as mean ± standard deviation (SD) of three independent experiments performed in triplicate and were analyzed by appropriate analysis of variance (ANOVA), followed by Duncan's multiple range test. Differences between groups were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Effect of n-hexane extract of *G. latifolium* on TBARS in brain of Wistar rats treated with QA.

Fig.1 shows that n-hexane extract of *G. latifolium* profoundly inhibited lipid peroxidation induced by QA in the cerebral tissues in a concentration dependent manner and this inhibitory effect was markedly different ($p< 0.05$) when compared with the control.

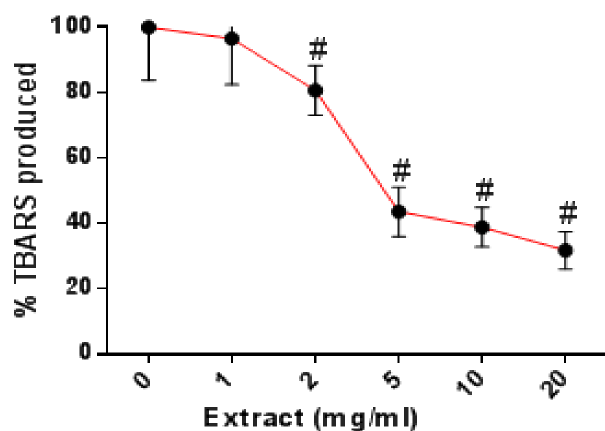

Fig. 1: Effect of n-hexane extract of *G. latifolium* on QA-induced lipid peroxidation in brain of wistar rats. Data are presented as mean ± SEM of three independent experiments carried out in different days.# Represent significant difference from control at $p < 0.05$.

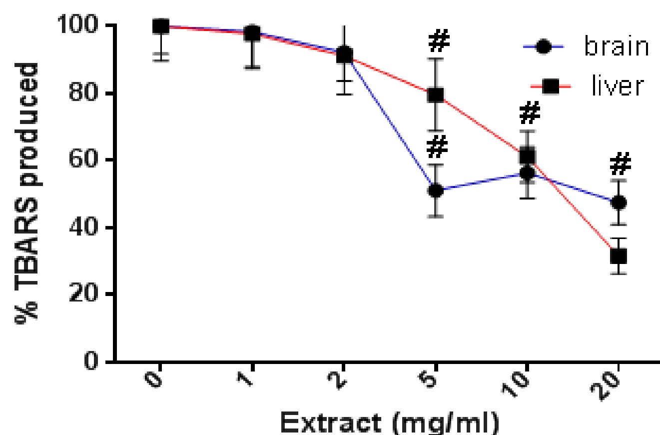

Fig.2: Effect of n-hexane extract of *G. latifolium* on SNP-induced lipid peroxidation in brain and liver of Wistar rats. Data are presented as mean ± SEM of three independent experiments carried out in different days. # Represent significant difference from control at $p < 0.05$.

Effect of n-hexane extract of *G. latifolium* on TBARS in brain and liver of wistar rats treated with SNP.

In the same fashion, extract of *G. latifolium* markedly inhibited lipid peroxidation induced by SNP in the hepatic and cerebral tissues of wistar rats with increasing concentration.

Effect of n-hexane extract of *G.latifolium* on the activity of delta-Aminolevulinic acid dehydratase in liver of wistar rat

Fig. 3 shows that *G. latifolium* exerted an increase in the activity of cerebral δ-ALAD. However, this increase was not significant ($p < 0.05$) when compared with the control.

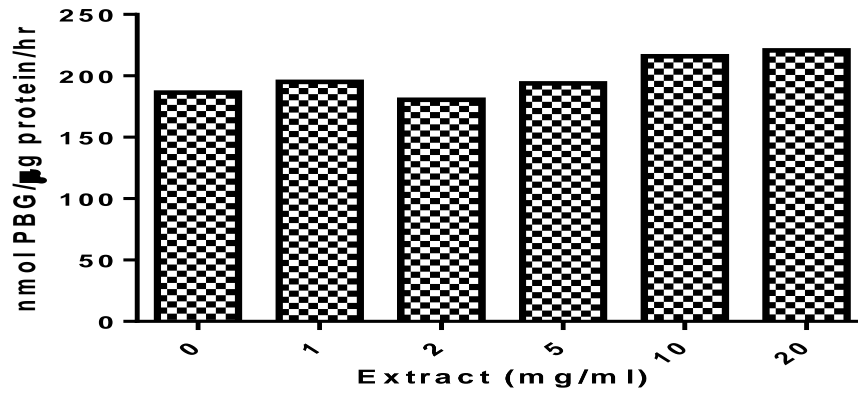

Fig.3: *Effect of n-hexane extracts of G. latifolium on the activity of δ-ALAD in Wistar rat liver. Data are presented as mean ± SEM of three independent experiments carried out in different days.# Represent significant difference from control at p < 0.05.*

Effect of n-hexane extract of *G.latifolium* on the activities of purinergic enzymes

The activities of both purinergic enzymes [5'-nucleotidase (Fig. 4) and NTPDase (Fig.5)] were significantly ($p < 0.05$) diminished in the cerebral and hepatic tissues with increasing extract concentration.

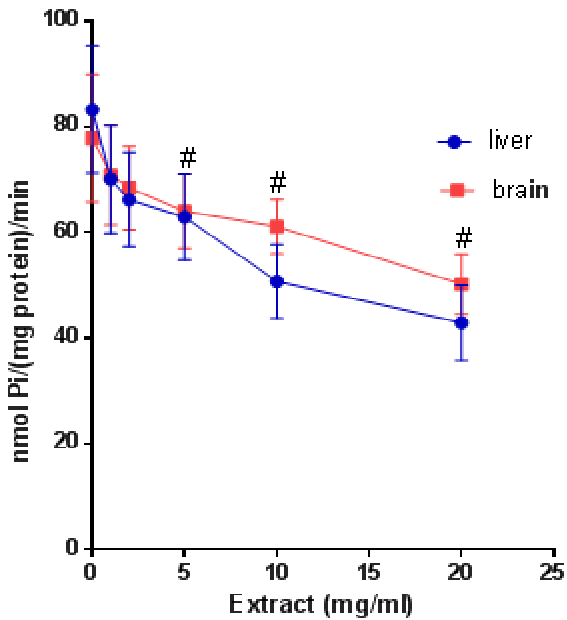

Fig.4: Effect of n-hexane extracts of *G. latifolium* on the activity of 5'-nucleotidase in Wistar rat brain and liver. Data are presented as mean ± SEM of three independent experiments carried out in different days. # Represent significant difference from control at p < 0.05.

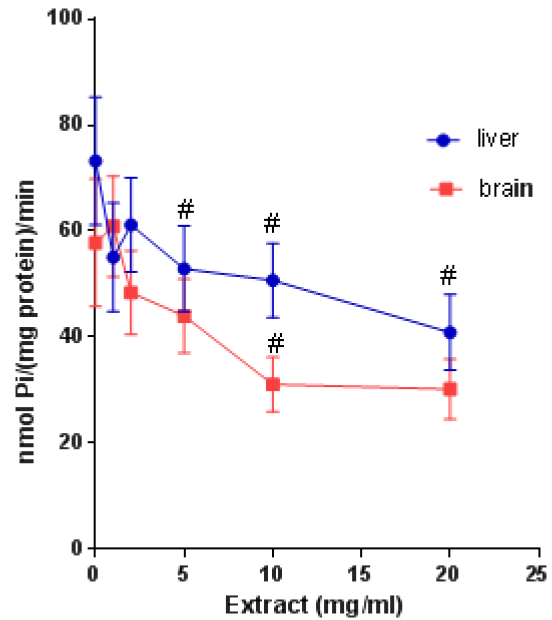

Fig.5: Effect of n-hexane extracts of *G. latifolium* on the activity of NTPDase in Wistar rat brain and liver. Data are presented as mean ± SEM of three independent experiments carried out in different days. # Represent significant difference from control at p < 0.05.

ROS include superoxide anion, hydrogen peroxide, hydroxyl radical and singlet molecular oxygen. A free radical is any chemical specie that can exist independently and possesses one or more unpaired electron (Cheeseman & Slater, 1993; Halliwell & Gutteridge, 1981). ROS constitute deleterious and harmful reactions in tissues and are capable of destroying tissues' biomolecules Lipid peroxidation mediated by free radical is considered to be primarily responsible for cell membrane destruction and cell damage leading to tissue injury and failure of the antioxidant defense mechanism (Ale, 2020).

Several reports have indicated that extracts of *G. latifolium* contain phytochemicals with potent antioxidant property and thus serve as candidate plant in the management of a number of ailments and degenerative conditions such as diabetes, malaria and ulcer (Lee *et al.,* 2011; Tona*et al.,* 2011; Chagas-Paula *et al.,* 2012).

Figures 1 and 2 show the effect of *G. latifolium* on lipid peroxidation induced by QA and SNP, respectively. QA and SNP treatment caused the peroxidation of brain lipids, but the administration of *G. latifolium*, extract exerted a significant (P<0.05) inhibitory effect on the formation of adduct of lipid peroxidation (Figure 1 and 2). In like manner, figure 2 also show that when hepatic tissue was subjected to oxidative stress induced by SNP, *G. latifolium* markedly (P<0.05) inhibited the peroxidation of hepatic lipids. One-way ANOVA revealed that, the inhibitory effect of *G. latifolium* was significant (P<0.05) with increasing concentration of extract tested. Furthermore, membrane lipids present in sub-cellular organelles are highly susceptible to free radical damage. Polyunsaturated lipids when reacted with free radicals can undergo oxidative degeneration which is a highly damaging chain reaction leading to both direct and indirect detrimental effects. From the foregoing, it is obvious that *G. latifolium* leaves offered protective effect against oxidative assaults induced by SNP and QA.

Moreover, quinolinic acid is a neurotoxin whose peroxidation is mediated by over activation of glutamate receptors (Ale, 2020). Reports have shown that sodium nitroprusside (SNP) elicits cytotxic effect through the release of cyanide and/or nitric oxide (NO) (Rauhala *et al*., 1998). NO has been implicated in the pathophysiology of strokes, traumas, seizures and Alzheimer's, and Parkinson's diseases (Ahmad *et al*., 2008). Besides, light exposure promote the release of NO from SNP through a photo-degradation process (Arnold *et al*., 1984), and data from the literature have shown that after the release of NO and SNP, $[NO\text{-}Fe\text{-}(CN)^{5-}]^{2-}$ is converted in to iron containing species (Loiacono and Beart, 1992). After the release of NO, the iron moiety may react with SNP, which could lead to the formation of highly reactive oxygen species, such as hydroxyl radicals via the Fenton reaction (Graf *et al*., 1984). The fact that *G. latifolium* extract inhibited SNP-induced lipid peroxidation may indicate that extracts possibly inhibited the breakdown of SNP to its constituents, thereby offering protective shield to both cerebral and hepatic tissues since the toxic constituents are presumably prevented from being released. It may also be that the extract exhibited an antagonistic properties against over activation of glutamate receptors by QA.

Herein, we also evaluated the effect of *G. latifolium* extract on δ-ALAD and purinergic enzymes (NTPDase and 5′-nucleotidase). δ-ALAD is an enzyme in heme biosynthetic pathway that catalyzes the condensation of two δ -aminolevulinic acid (ALA) molecules to porphobilinogen, a precursor of heme (Jaffe, 1995). δ-ALAD inhibition may impair heme biosynthesis (Sassa *et al*., 1989) and this can result in the accumulation of ALA, which may affect the aerobic metabolism and as well constitute some prooxidant activities (Becharaet *al.,* 1993). Figure 3 reveals that the n-hexane extract of *G. latifolium* increased the activity of the enzyme in a concentration dependent manner and at concentration. However, the increase is weak and not significant (P<0.05) when compared with the control. This implies that the extract does not constitute a vicious effect on heme biosynthesis.

Studies revealed that the roles of purines might be altered in pathological states in the body (Mutafova-Yambolieva and Durnin, 2014). Purinergic enzymes (NTPDase and 5′-nucleotidase) contribute to the maintenance of physiological levels of extracellular ATP and adenosine. NTPDase is a membrane-bound enzyme that hydrolyzes ATP and ADP to AMP, which is subsequently converted to adenosine by 5′-nucleotidase (Robson *et al*., 2006; Strater, 2006). Thus, they could constitute a considerable point of regulation in several physiological and pathological conditions (Oses *et al*., 2004; Agteresch *et al*., 1991). It is therefore of note that purinergic mechanisms are involved in several pathologies including brain trauma and ischemia, neurodegenerative diseases such as diabetes (Burnstock, 2013), neuro-inflammatory reactions (Burnstock, 2008) and hypertension (Burnstock, 2006; 2009). In fact, studies reported that purinergic mechanisms are potential therapeutic targets for the treatment of neurological (Engel *et al*., 2016) and degenerative disorders (Koles *et al*., 2005, Woods *et al*., 2016). As presented in Figure 4 and 5, the activities of the enzymes were markedly (P<0.05) reduced by the n-hexane extract of *G. latifolium* with increasing concentration. The reduced NTPDase and 5′-nucleotidase activities observed in this study could be consequent to the modulatory effect of the extract to a healthy physiological level by counteracting pathophysiology of diseases that can provoke high level of nucleotides in the circulation.

CONCLUSION

In conclusion, *G. latifolium* leaves extract can be considered a potent antioxidant. It elicited a modulatory effect on the activities of δ-ALAD and purinergic enzymes within a pharmacological level. In any case, it is rational to suggest that *G. latifolium* could be an ideal intervention in the management of degenerative diseases and in search of a promising novel purinergic targeting drugs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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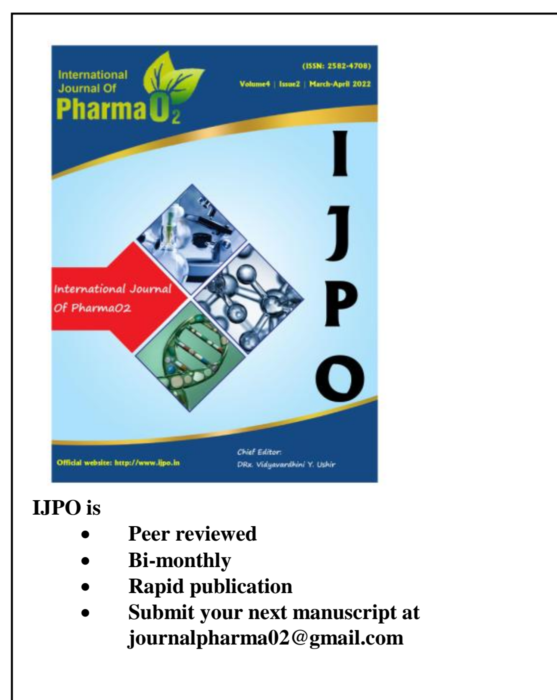

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