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Oil extract from *Gongronema latifolium* leaves exhibit anti-diabetic and anti-ulcer activities

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Air-dried, pulverized leaves of *Gongronema latifolium* were subjected to step wise fractionation using first, ethanol and subsequently fractionation of the dried ethanol extract using solvents of increasing polarity, n-hexane, chloroform, ethylacetate and ethanol. Column fractionation of the n-hexane extract using graded solvent mixture of n-hexane and ethylacetate in specified ratios, yielded four fractionates, one of which F2 was an oil. The F2 (oil) fraction was characterized by gas chromatography/mass spectral analysis. The effects of this oil fraction on alloxan- induced diabetes mellitus and indomethacin - induced ulceration were studied. The results obtained reveals that oil extract evoked significant inhibition of both alloxan- induced diabetes and indomethacin- induced ulcer in rats. The oil on analysis revealed content of essential fatty acyl esters and aromatic dicarboxylic acids, all of which are implicated in inhibiting hyperglycaemia and ulcer formation probably through oxidative reaction or through production of prostaglandins.

Key words: *Gongronema latifolium*, essential fatty acids, diabetes mellitus, ulcer, prostaglandins.

INTRODUCTION

Extractable oil, an important nutritive component of plants, have not been properly projected and utilized. Fats and oils are naturally occurring organic compounds of plants whose function is mostly to supply energy and serve as structural components of membranes and in pharmaceuticals as drug delivery (Ajalli, 2004; Garret and Grimsham, 2005; Ezekwe, 2013; Odo et al., 2013). The amount and type of fat in diet (solid or oil) have important implication because a diet containing large amount of saturated fat is linked to an increase in risk of atherosclerosis and subsequent heart disease and stroke (BMA, 2002). On the other hand, animals maintained on fat free diet develop poor growth, poor wound healing and dermatitis (Ajalli, 2004).

Plants essential oils and their components have been associated with biological activities such as possessing antimicrobial and anti pests (Dongmo et al., 2007; Amvam Zollo et al., 1998), anti-oxidant (Lemos et al., 2006), anti-flammatory, anti-ulcer (Olivierra et al., 2004; Siani et al., 1999) activities and protection against cardiovascular disease (Ezekwe, 2013; Odo et al., 2013). Linoleic acid component of oils, is the precursor of prostaglandins which act as hormone to protect the lining of the stomach against ulceration, lowers blood pressure and stimulate contraction of uterine walls (BMA, 2002). However, other works suggest that excess of polyunsaturated fats is central to the development of degenerative diseases. They are universally toxic to energy producing systems and act as “misleading signal” channelling cellular adaptation down self-defeating pathways and diabetes is one of the terminal diseases that can be caused by the polyunsaturated vegetable oils (Peat, 1996). Even impaired insulin access to tissues may be one of the initial consequences of elevated free fatty acids (Arbeeny et al., 2001; Garrett and Grimsham, 2005).

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It is based on these conflicting views that this work was undertaken in order to ascertain if dietary oils from a vegetable could exhibit protection against some chronic disease such as ulcer and diabetes. And especially, as it has been observed by the World Health Organization (WHO, 2003; Ezekwe, 2013; Odo et al., 2013), that insufficient consumption of fruits and vegetables, a major source of these oils, is among the major cause of diseases of chronic conditions such as diabetes mellitus, cardiovascular diseases, cancers, especially of the gastrointestinal tract.

Hence, the advocacy by the WHO promoting increase in fruit and vegetable intakes. *Gongronema latifolium* is one of the perennial herbal plants of Nigeria that have aromatic properties of important medicinal value (Arbeeny et al., 2001). It is listed among the medicinally important leafy vegetables of South West Nigeria (Ayodele, 2008) and as Africa leafy vegetables (Smith and Eyzaguirre, 2007; Siani et al., 1999). It has been implicated with anti-diabetic and anti-oxidant properties (Ezekwe, 2005; Ugochukwu and Babbady, 2003; Amvam Zollo et al., 1998), anti-inflam-matory properties (Esterbauer and Puhl, 1991) and intestinal muscle relaxation (Gamaniel and Akah, 1996; Cavanagh, 2007). The anti-diabetic and anti-ulcer effects of the oil extract, from the leaves of this plant, were determined on rats.

MATERIALS AND METHODS

Plant materials

Leaf samples of *G. latifolium* were air-dried, pulverized and stored in plastic containers at -10°C.

Animals

Thirteen (13) albino mice weighing between (19 to 30 g) were purchased from the Departmental Animals House, University of Nigeria, Nsukka. They were housed in metal cages under standard conditions of 12 h light/dark cycles, fed pelleted feed and water *ad libitum.*

Rats

Inbred Wistar albino rats weighing (150 to 250 g) were purchased from Departmental Animal House and housed under same conditions as the mice.

Extraction procedure

Crude ethanol extract

Air-dried pulverized leaves (1 kg) were macerated in 5.0 L of 96% ethanol for 48 h. The filtrate from Whatman No. 1 filter paper was dried at 40°C and the Crude Ethanol Extract (CEE) and stored for further use.

Fractionation of crude ethanol extract (CEE)

The dried CEE (75 g) was adsorbed on silica gel G (1:2 w/w) and extracted with n-hexane. The fraction was dried and stored for biochemical determinations.

Fractionation of n-hexane fraction

The dried n-hexane fraction (20 g) was chromatographed on silica gel (70 to 230) mesh packed into a glass column (4 × 120) cm, with a bed of 60 cm height. Graded solvent mixtures of n-hexane and ethylacetate were used for the elution that is, n-hexane, n-hexane: ethylacetate 19:1, 9:1. Aliquots of 50 ml were collected and concentrated. Similar fractions were pooled by thin layer chromatography and on further concentration yielded fractions 1 and 2.

F1 - white waxy substance,
F2 - yellowish brown oil.

Animal studies

Determination of the effect of F2 on alloxan - induced diabetes mellitus

Four groups of five rats were used representing: 1) Normoglycaemic, 2) diabetic F2-treated, 3) diabetic glibenclamide-treated, and 4) diabetic non-treated. Diabetes was induced by the method of Abdel-Hassan et al. (2000) by i. p. administration of alloxan (150 mg/kg b. w.).

Diabetes was confirmed after 72 h of persistent hyperglycaemia above 300 mg/kg b. w. as shown previously by Al-Hadar et al. (1994). Blood glucose levels were monitored at intervals of 0, 1, 3, 6, 12 and 24 h. The % reduction in blood glucose is computed after 24 h.

Determination of the effect of F2 (oil on indomethacin-induced ulceration)

The method of Uridishani et al. (1979) was used. Three groups of four rats were used namely: GP 1 - saline, Gp 2 - F2 and GP 3 - ranitidine. Food was removed from the rats (12 h) and water just before the experiment. The different groups received their drugs (extract) 30 min before oral administration of 40 mg/kg indomethacin. After 72 h, rats were sacrificed in ether chamber and their stomachs excised, dissected, washed, fixed in formal saline and mounted on slab. Ulcer crater or wounds were counted using a magnifying (×10) lens and the mean ulcer indices computed.

Structural elucidation of F2

F2 was analysed by ‘gas chromatograph/mass' spectral analysis using agilent 5973N mass selective detector coupled to Agilent 6890N gas chromatograph with specified parameters. Column size (30 × 0.2 mm, film thickness 0.25 μm). Operating conditions - carrier gas helium with a flow rate of 2 ml/min, column temperature (60 to 275° at 4°C/mm), injection and detector temperature (280°C), injector volume (2 μl), split ratio (1:5).

The MS operating parameters were as follows: ionization potential (70 eV), ionization current (1A), ion source temperature (200°C) and resolution of 1000. Identification of F2 was based on comparison of the retention times and computer matching of MS fragments with NISTO2 library.

Statistical analysis

The results obtained were analysed by SPSS version 18 using one way analysis of variance (ANOVA) and subjected to Fischer LSD

Table 1. The chemical composition of sample F2.

Compound	RT (min)	Molecular weight	Molecular formula	MS Fragment ions
Ethyl hexadecanoate (palmitic acid ester)	18.25	284	$C_{18}H_{36}O_2$	284, 255, 241, 157, 151, 88, 55
Ethyl 9, 12 - octadecadienoate (linoleic acid ester)	21.03	308	$C_{20}H_{36}O_2$	308, 277, 263, 250, 164, 149, 135, 121, 108, 95, 81, 67, 55
(Z,Z,Z) ethyl 9, 12, 15 - octadecatrienoate (linolenic ester)	21.08	306	$C_{20}H_{34}O_2$	306, 277, 261, 250, 237, 203, 191, 173, 149, 135, 121, 108, 95, 79, 67, 55
Ethyl oleate (oleic acid ester)	21.15	310	$C_{20}H_{38}O_2$	310, 284, 264, 246, 235, 222, 180, 166, 152, 137, 123, 110, 97, 83, 69, 55
Ethyl octadecanoate (stearic acid ester)	21.68	312	$C_{20}H_{40}O_2$	312, 269, 213, 157, 101, 88, 83, 73, 61, 55
Diisoctyl - 1, 2 - benzenedicarboxylate	26.82	390	$C_{24}H_{38}O_4$	390, 279, 167, 149, 132, 113, 93, 83, 71, 57

Table 2. Effect of F2 fraction on diabetes mellitus.

Treatment group	Dose	Mean blood glucose concentration (mg/100 ml)/time						Maximum % reduction
		O h	1 h	3 h	6 h	12 h	24 h/time	
Normoglycaemic	5 ml/kg	119.70[a] ± 7.0	109.00 ± 8.0	93.00 ± 9.0	78.70 ± 7.0	74.00 ± 4.0	55.00 ± 5.0	53.78%
Diabetic F2- treated	100 mg/kg	429.75 ± 30.3	390.50 ± 49.3	370.00 ± 40.1	212.25 ± 43.1	137.50 ± 22.1	83.75 ± 12.9	80.51
Diabetic glibenclamide treated	100 mg/kg	460.00 ± 23.0	257.0 ± 22.0	200.00 ± 10.0	156.00 ± 24.0	118.0 ± 28.0	68.0 ± 9.0	85.22
Diabetic untreated (negative control)	5 ml/kg	356.00 ± 23	409.50 ± 38.8	363.5 ± 27.0	382.50 ± 18.0	341.29 ± 22.0	270.0 ± 19.0	24.60

P<0.05 against negative control.

post HOC. Results were expressed as mean ± SEM. Differences between means were considered significant at $P < 0.05$.

RESULTS

Table 1 shows that F2 is composed of six esters of fatty acids, of which two are essential fatty acids (linoleic, linolenic acids), one mono unsaturated (oleic acid), two saturated (stearic and palmitic acids and an aromatic fatty acid). Diisoctyl 1, 2 - benzenedicarboxylate. The standard anti-diabetic drug glibenclamide inhibited hyperglycaemia significantly ($P < 0.05$). The F2 fraction equally inhibited hyperglycaemia significantly ($P < 0.05$). The maximum reduction of hyperglycaemia in the three treated groups of animals was 56.1, 80.51 and 85.22%, respectively (Table 2). The glibenclamide significantly ($P < 0.05$) lowered the blood glucose level over time. The fall in blood glucose was more prominent

Table 3. Effect of F2 on indomethacin- induced ulcer in rats.

Group	Dose	Mean ulcer index	% inhibition of ulcer
Saline	3 ml	6.10 ± 0.94	
F2-treated	100	*0.77 ± 0.27	87.38
Ranitidine	100	*0.75 ± 0.26	87.70

*P < 0.05.

from the 3rd hour of the experiment. Similarly, the F2 isolate (the oil fractions) significantly (P < 0.05) reduced hyperglycaemia. The drop in blood glucose was more prominent between the 5 and 6th hours. The n-hexane extract lowered blood glucose but it was not significant (p > 0.05). The standard drug, ranitidine, significantly (P < 0.05) hindered ulceration of rat stomach mucosa (Table 3). Similarly, F2 fraction hindered ulceration in rat stomach significantly (P < 0.05). The inhibition of ulcer by the n-hexane fraction at 400 mg/kg was below that of the F2 and ranitidine.

DISCUSSION

The phytochemical screening of the crude ethanol extract revealed an abundance of phytochemicals in the crude ethanol extract many of which have been implicated in disease remedies. Shimizu et al. (2001) and Williams et al. (2007) reported that triterpenoids of *Gymnema inodorum* inhibited the absorption of glucose while Abdel-Hassan et al. (2000) demonstrated that alkaloids and saponins in aqueous extract of *Citrullus colocythis* exerted hypoglycaemic effect on diabetic rabbits. Flavonoids of *Equisetum myriochaetum* have also been implicated in reduction hyperglycaemia in streptozotocin diabetic rats (Ezekwe, 2005; Williams et al., 2007; Ezekwe, 2013; Odo et al., 2013). The result from this work, showed that hyperglycaemia induced by alloxan, was significantly (P < 0.05) inhibited by the oil extract, (F2), of *G*. *latifolium*. The magnitude of inhibition was 56% for n-hexane, 80% for the oil (F2) and 85% for the standard drug. This work also confirms the family relationship between *G*. *latifolium* and *Gymnema sylvestre* as both of them significantly reduced hyperglcaemia. Studies carried out on *G*. *sylvestre* showed that oil extracts possessed hypoglycaemic activity (Punitha et al., 2005). GC/MS analysis revealed that two of the components of F_2 are essential oils (linoleic and linolenic acids), in addition to an aromatic oil, saturated and mono unsaturated fatty acyl esters, which tend to suggest that these components, must have contributed to the total reduction in blood glucose level. Such complications as nerve and kidney disorders in diabetes are known to improve when evening rose oil, a source of r-linolenic acid was administered to patients (Day, 1998) but no direct link between dietary oil and protection from diabetes mellitus have been established yet. However, (Peat, 1996) indicated that polyunsaturated fats were central to the cause of degenerative diseases such as diabetes mellitus, whereas coconut oil proved to be protective. This work also showed that the F2 oil evoked a significant (P < 0.05) inhibition of ulcer in drug-induced ulcer.

The ulcer inhibition was comparatively close between the standard drug (87.70%) and the F2 (87.38%). This work vitiates the studies carried out on *G*. *latifolium* by Ogunwande et al. (2007) who showed that *G*. *latifolium* contained essential oils which had anti-microbial activity. Essential oils facillitate gastroprotection as a result of tendency to generate prostaglandins from arachidonic acid, a product of action of cyclooxygenase on linoleic acid (Snowdon and Philips, 1985; Amvam Zollo et al., 1998). Several works have implicated prostaglandin in inhibition of ulcer as a result of bicarbonate mechanism of gastric protection by the mucosa (Bottling and Salzamnn, 1974; Lauritzen et al., 2001). An important inference from this study is that the extract was non-toxic even at the highest dose level (5000 mg/kg b. w.). This therefore does not place any limitation to the use of the vegetable.

In conclusion, the oil extract (F2) from *G*. *latifolium* was effective in protecting against diabetes and ulcer, two pathological conditions of degenerative nature. These two conditions, usually attributed to oxidative reactions, might suggest that the oil extract functioned as antioxidant. This possibility is under investigation. Hence, oil extract of *G*. *latifolium* did not exhibit deleterious effects in the animal studied but rather inhibited hyperglycaemia in alloxan-induced diabetic rats and protected against ulcer in indomethacin-induced ulcerative rats.

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