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Bioactive compounds from GC-MS analysis in methanolic extract of *Gongronema latifolium* inhibits aluminium chloride induced oxidative stress in rat spermatozoa *in vitro*

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Abstract

This study investigated the effect of *Gongronema latifolium* extract on aluminum chloride-induced sperm oxidative stress. Spermatozoa were obtained from the epididymis and dispersed in a buffer consisting of tris-(hydroxymethyl)-aminomethane, citric acid anhydrous and D (−) fructose (TCF buffer) and incubated with *Gongronema latifolium* alone (0.8 mg/ml), AlCl3 alone (50 mM) and *Gongronema latifolium* (0.2 – 0.8 mg/ml) + AlCl3 (50 mM) repectively, for 3 h at 32 0C. We found that exposure to aluminum chloride alone led to significant increase in lipid peroxidation (LP) 14.41±1.18µM and protein carbonyl content 19.21±0.9nM, but significant decrease on glutathione content 5.32±3.11mM. When *Gongronema latifolium* was added in the incubation medium, it improved and protected spermatozoa against the harsh effect of AlCl3 on sperm cells. Bioactive compounds detected from GC-MS analysis also revealed the presence of squalene, ascorbic acid, gamma tocopherol, phthalic acid,carbazic acid amongst others. This study showed that *Gongronema latifolium* could protect spermatozoa against AlCl3 induced sperm damage.

Keywords: *GongronemaLatifolium*; Aluminum Chloride; Oxidative Stress; Spermatozoa; GC-MS; Glutathione

1. Introduction

Aluminium chloride is abundant in the environment and can be easily contaminated by humans. Humans ingest through food, water and other sources an average of 7600 µg/day of aluminiumfrom drinking water and food [1]. Male reproduction system may be affected badly by Al exposure [2]. Due to these adverse effects non-toxic cheap plant extract are investigated one of such plants is *Gongronema latifolium.*

Gongronema latifolium is a non woody plant from the family ofAsclepiadaceae. It is largelywidespread in tropics, especially Africa and South America, with a lightrepresentationin Northern and South Eastern Asia [3]. In South Easternand South Western Nigeria, *Gongronemalatifolium*is commonly called “utazi” and “arokeke”, respectively, and is primarily used as spice and vegetablesin traditional medicine [4].

2. Material and methods

2.1 Chemicals

Aluminum chloride, dinitrophenylhydrazine, tris-(hydroxymethyl)-aminomethane, citric acid, fructose, 5, 5′-Dithiobis-2-nitrobenzoate, phosphoric acid, sodium hydroxide were obtained from Sigma-Aldrich (Germany). All other chemicals are of analytical grade and were source locally.

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2.2 Preparation of Plant Extract

The leaves of *Gongronema latifolium*were collected fresh, shade-dried and powdered. This was then subjected to extraction with methanol for 3 days. The extract was later filtered and evaporated to form a thick dark paste. This was stored in the refrigerator for further use.

2.3 Animals

Wistar albino male rats, 5–6 weeks old, (150-200 g) were used for the study. Animals were kept in non-aluminum cages under a well-regulated light:dark (12 hr:12 hr) schedule or cycle at 25 0C and were given standard commercial laboratory chow and tap water ad libitum.

2.4 Collection and incubation of epididymal spermatozoa

Collection and incubation of epididymal spermatozoa was according to [5] and [6]. Stock concentration of plant extract *Gongronema latifolium*(5 mg/ml) was also made. Spermatozoa were dispersed in TCF buffer and were incubated with *Gongronema latifolium*(1 mg/ml) alone, Aluminium chloride alone (50 mM) and *Gongronema latifolium*(0.2-1 mg/ml plus Aluminium chloride (50 mM) for 3 h at 32 ^{0}C.

2.5 Biochemical assays

At the end of the incubation time, sperm cell suspensions were homogenised at 4 ^{0}C for 10 s and centrifuged at 800 g for 10 min and the supernatant was used for biochemical assays. The glutathione (GSH) content in spermatozoa was determined by the method of [7] and lipid peroxidation assay was according to [8], protein carbonyl content assay was carried out according to the method of [9].

Gas chromatography- mass spectrophotometry analysis (GC-MS Analysis)was carried out according to the standard manual guidelines and operations.

2.6 Statistical analysis

Statistical analyses were carried out using Student's t-test using SPSS (student version 7.5; SPSS Inc., Surrey, UK), and values <0.05 were considered statistically significant.

3. Results and discussion

The effects of different concentrations of *Gongronema latifolium* extract (0.2 – 0.8 mg/ml) on rat sperm glutathione, lipid peroxidation and protein carbonyl content after incubation for 3 h is presented in Table 1. Results showed decrease in glutathione content in the $AlCl_3$ (50 mM) alone, but this decrease was seen to be reversed in the *Gongronema latifolium* extract (0.2 – 0.8 mg/ml). Results for the lipid peroxidation revealed increased levels of LPO content in the $AlCl_3$ (50 mM) alone, but this decrease was seen to be decreased in the *Gongronema latifolium* extract (0.2 – 0.8 mg/ml).

Table 1 Levels of Lipid peroxidation, Glutathione and Protein Carbonyl Content in $AlCl_3$ Induced rat Spermatozoa, of the different experimental groups (n = 5) after 3 h of incubation

	Glutathione (GSH)	**Lipid peroxidation LPO**	**Protein carbonyl content (PCC)**
Control	13.40 ± 4.08 mM	4.69 ± 3.78 µM	9.89 ± 0.49 nM
$AlCl_3$(50 mM) alone	5.32 ± 3.11 mM	14.41 ± 1.18 µM	19.21 ± 0.9 nM
g.latifolium 0.8 mg/ml	12.22 ± 1.44 mM	5.33 ± 3.58 µM	10.11± 1.48 nM
0.2 mg/ml + $AlCl_3$	15.91± 0.99 mM	9.36 ± 2.08 µM	13.43± 3.59 nM
0.4 mg/ml + $AlCl_3$	12.83± 0.97 mM	7.16 ± 1.48 µM	12.43± 4.11nM
0.6 mg/ml + $AlCl_3$	6.28± 0.19 mM	9.43 ± 0.79 µM	16.50± 1.32 nM
0.8 mg/ml + $AlCl_3$	8.56± 0.51 mM	10.66 ± 0.11 µM	14.16± 4.09 nM

Values are mean ± SD n= 5 determination

Results for the protein carbonyl content revealed increased levels of PCC content in the $AlCl_3$ (50 mM) alone, but this increase was seen to be decreased in the *Gongronema latifolium* extract (0.2 – 0.8 mg/ml).this could be due partly to the fact that *Gongronema latifolium* extract has bioactive ingredients as revealed in the GC-MS acting as antioxidants.

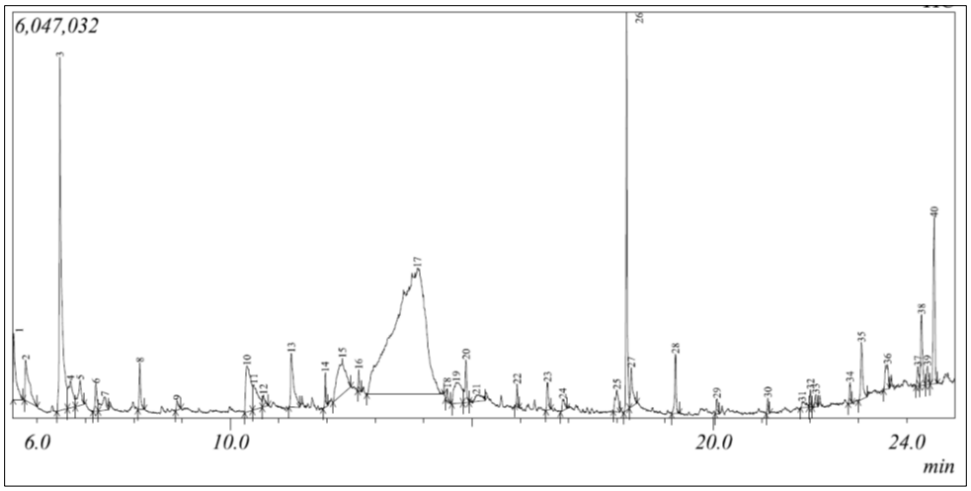

Figure 1 GC–MS chromatogram of compoundsinmethanolic extract of *Gongronemalatifolium*

Table 2 Compounds detected in GC-MS analysis of Methanolic Extracts of *Gongronemalatifolium*extract

Peak no	Name of compound	Retention time	Area%
1	2-methyl-2-hexene	5.515	1.82
2	Diethyl-diethyl-silane	5.763	1.91
3	4-ethyl cyclohexanone	6.469	9.29
4	3-ethyl-2-methyl-3-pentanol	6.690	1.47
5	1-methyl-2-(1-methylethyl)-benzene	6.890	0.98
6	1,1-diethoxy-2-hexene	7.215	0.62
7	3-isopropenyl-5,5-dimethylcyclopentane	7.399	0.80
8	Trimethyl silyl-2-butynoate	8.125	0.78
9	5-methyl-2-(1-methylethyl)-cyclohexanol	8.895	0.22
10	5-methyl-2-(1-methylethyl)-phenol	10.342	2.80
11	1-adamantylmethyl-3-fluorobenzoate	10.483	1.53
12	1-(1-cyclohexen-1-yl)-pyrrolidine	10.683	0.33
13	N,N,2,4-tetramethylbenzenamine	11.262	2.03
14	caryophylline	11.964	0.49
15	1,4-anhydro-d-mannitol	12.317	3.50
16	Naphthalene	12.657	0.28
17	Ethyltrimethylolmethane	13.869	47.50
18	2-azidomethyl-1,3,3-trimethyl-cyclohene	14.487	0.22
19	1,2,3,4,5-cyclohexanepentol	14.676	1.86
20	Tricyclohexylmethane	14.877	0.93
21	4-O-methylmannose	15.092	0.59

22	Acetylphytol	15.936	0.31
23	Palmitic acid	16.562	0.54
24	Ascorbic acid	16.883	0.38
25	Methyl 8,11,14-heptadecatrienoate	17.999	0.82
26	Citronellol	18.201	6.33
27	5-benzyl-2-methyl-2-(4-nitrophenyl)-2,3-dihydro-[1,3,4]thiadiazole	18.296	1.13
28	Hexadecimal	19.212	1.17
29	3-beta-acetoxy-bisnor-5-choleric acid	20.073	0.28
30	Nonadecane	21.125	0.25
31	Carbazic acid	21.833	0.31
32	Octacosane	22.005	0.28
33	Phthalic acid	22.116	0.23
34	Henicosane	22.825	0.31
35	Globutol	23.061	1.35
36	2,6,10-trimethyl dodecane	23.601	0.89
37	2,3,4,5-tetraphenyl-2,4-cyclopentadien-1-yl benzene	24.231	0.41
38	Bicycle [4.4.0]dec-2-ene-4-ol	24.305	1.53
39	Gamma tocopherol	24.430	0.42
40	Squalene	24.566	3.09

There are many compounds detected in *Gongronema latifolium* GC-MS analysis which include 4-ethyl cyclohexanone has a peak number of of 3 retention time (RT) 6.469 and % area of 9.29. Another important biocompound in *Gongronema latifolium* extract was 1,4-anhydro-d-mannitol with a % area of 3.5 less than that of 4-ethyl cyclohexanone. Ethyltrimethylol methane has a peak number of 17 RT of 13.86 and % area of 47.50 is the most abundant in *Gongronema latifolium.* Others with moderately higher % areas are citronellol with peak number 26, RT of 18.20 and % area of 6.33 and squalene a precursor of alpha tocopherol has a peak number of 40 RT 24 and % area of 3.09.

4. Conclusion

In conclusion, the effects of *Gongronema latifolium* extract may be partially due to its biocompounds detected in the GC-MS analysis.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest.

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