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Histological Effects of *Abelmoschus esculentus* Extract and its Isolated Flavonoid Glycosides and Triterpene on Mice Heart

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Abstract:- The study obtained the crude extract of Abelmoschus esculentus (Okra) fruits, isolated the compounds from the extract and characterized the structures of the isolated compounds. It also investigate the histological effect of the crude extracts and its isolated compounds on mice heart. These helps in providing information and facts on the cardiovascular activities of A. esculentus (Okra) fruit extract and its isolated compounds on mice heart. The Abelmoschus esculentus fruits were collected, identified, dehydrated (air dried), and pulverized. The grinded plant material was macerate with 100% ethanol for 72 hours. Separation of the phytochemicals from the extract was achieved by repeated open chromatographic procedures. The isolated compounds were subjected to spectroscopic analysis such as mass spectrometry (MS), ultraviolet (UV) and nuclear magnetic resonance (NMR). The histological effects of the crude extracts and its isolated compound was evaluated, the animals were divided into digoxin (reference or positive control), distilled water (negative control) and treatment (administered the crude extract or isolated compounds) groups. A dose of 1 mg/kg body weight of digoxin and a dose of 10 mg/kg body weight of the crude extract or isolated compounds were administered daily intraperitonealy for ten days. The animals were sacrificed on the eleventh day using diethyl ether as anaesthetic agent with their heart harvested and preserved using 10 % formalin followed by the histological studies on the isolated organ (heart). Three compounds from two different classes of compounds were isolated and elucidated from A. esculentus (two flavonoids and one triterpene). Also demonstrated cardiovascular activities of Abelmoschus esculentus extract and its isolated compounds on mice heart. The flavonoid glycosides (Quercetin glycoside and Quercetin diglycoside) present in A. esculentus preserved the cardiac muscle architecture similarly to digoxin the classical cardiac glycoside except Ursene-3-O-β-D-Glucopyranoside which shows dilatation of the cardiac muscle and some degenerative nuclei.

Keywords:- *Isolation, Elucidation, Histological Effect, Cardiovascular Activities, Abelmoschus esculentus, Flavonoid, Triterpene.*

I. INTRODUCTION

Cardiovascular diseases (CVD) is a leading source of morbidity and mortality in the worldand had been proposed to remain so, with a projection of 23.6 million deaths worldwide by 2030 [1].

Drugs with digitalis-like effect (especially, digoxin) are used to manage congestive heart failure and there is support for the continuous use of this class of drug especially in heart failure with reduced ejection fraction and also in atrial fibrillation[2]. However, digoxin has a very narrow therapeutic window, thus necessitating continuous search for more effective and safer compounds with digitalis like effects especially from medicinal plants. In the developing countries of the world, there is heavy reliance on medicinal plants for treatment of many ailments, CVD inclusive, because of the affordability, accessibility, availability, cultural acceptability among others.

Okra *(Abelmoschus esculentus*), orginated from Malvaceae family, is one of the most extensively consuming species of Malvaceae (America Botanical Council, 2012). Due to its comestible fruits property, it is widely cultivated in subtropical and tropical region of the earth and harvest while immature, fresh and young. *A. esculentus* is about to 6 feet in height, with robust stems, serrated, long, broad and deeply-lobed leaves. The delicate yellow flowers are marked with purple or red colour near the base [3].

Many phytochemical constitutents have been reported from *A. esculentus* which includes (3β)-9,18-dihydroxyolean-12-en-3-yl acetate, (3β, 21β)-19,21-epoxylup-20-en-3-yl acetate[4]. Polyphenolics group of compounds like flavonoids (quercetin-3-O-sophoroside) hydroxycinnamic derivatives[5], isoquercitin and 5,7,3′,4′tetrahydroxyflavonol-3-O-[β-d-rhamnopyranosil (1→2)] β–d-glucopyronoside[6]. Other includes rutin [7], 5, 7, 3′, 4′- tetrahydroxy- 4″- O-methyl flavonol -3- O- β- D- glucopyranoside, Procycanidin B1 and B2 [8], oligomeric proanthocyanidins[9].

Abelmoschus esculentus has been found industrially and medically useful. Its mucilage discovers it usefulness as suspending agents in some pharmaceutical industries[10]. It medicinal uses includes as blood volume expander or plasma replacement[11]. Okra also help in inhibiting cancer cell

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multiplication due to the presence of Lectin [12]. *A esculentus* was also reported to have rhamnogalacturonan polysaccharides which helps in the treatment of stomach ulcers[13]. Okra was found to be effective at binding bile acids with 34% as effective as cholestyramine [14]. *Abelmoschus esculentus* also helps in the treatment of hyperlipidaemia and diabetes [15], and also aids cardiovascular disease treatments[4]. However, there have been little reports in literature on the cardiovascular properties of the Okra extract or its isolated compounds, prompt this study".

II. METHOD

A. Isolation of Compounds.

The Collection of Abelmoschus esculentus fruits were done at the central market of the Obafemi Awolowo University and the identification was done by the Curator of the Faculty of Pharmacy, Obafemi Awolowo University, Nigeria (Mr. Ogunlowo A. A). Specimen number FPI 2221 was tagged to the fruit specimen in the herbarium. The friuts were dried (air-dried) at room temperature and pulverized. The grinded fruits which weighed 1.0 kg was extracted (maceration) with 100 % methanol of about 5liters for 72hours. The extracted crude (filterate) was concentrated in vacuo to give 164.8 g of the crude extract. The crude extract was subjected to numerous purification (chromatographic processes) to give **compound 1** (0.0085g) which is yellow in colour, with Rf -0.52, **compound 2** (0.034g) which is brown in colour with Rf -0.44 and **compound 3** (0.024g) which is an white amorphous powder, with Rf - 0.58..

B. Identification and Elucidation

Elucidation of active phytochemicals in A. esculentu was archived using open column chromatography and size exclusion chromatography. Silica gel (ASTM 230–400 mesh, Merck and Sephadex LH-20 (Pharmacia) were used for open column and size exclusion chromatographies respectively. Column eluates were analyzed by Thin Layer Chromatography (TLC) observed at room temperature. Mobile phase was made of EtOAc: MeOH: H_2O: AcOH, in the ration of 10:2:1:0.2. The visualization TLC plates (resultinspots) were performed under UV light (wavelenth 254 nm) and detected with the use of 10% H_2SO_4 in CH_3OH.. ^1H and ^{13}C NMR spectra (for both 1D and 2D experiments) were acquired on the Bruker AV400 Spectrometer on both 300 and 75 MHz for ^1H and ^{13}C spectra respectively at the School of Pharmacy and Biomolecular Sciences, Faculty of Science, Liverpool John Moores University, UK. MS analyses were done on an Agilent TOF spectrometer at the School of Chemistry and Physics, Faculty of Science, Pietermaritzburg Campus, Kwazulu-Natal University, South Africa**.**

C. Histology Study.

Both male and female mice with weight range of 18-22 g were used in this study. All animal were kept in the animal house (Department of Pharmacology, Faculty of Pharmacy). All animal were treated according to the standard procedures of the animal use. The animals were divided into six namely; digoxin (reference or positive control), distilled water (negative control) and four treatments (administered the crude extract and three isolated compounds) groups. A dose of 1mg/kg body weight of digoxin and a dose of 1mg/kg body weight of the crude extract or isolated compounds were administered daily intraperitonealy for ten days. The animals were sacrificed on the eleventh day using diethyl ether as anaesthetic agent with their heart harvested and preserved using 10 % formalin followed by the histological studies on the isolated organ (heart**).**

III. RESULTS AND DISCUSSIONS

A. Structural Elucidation of Isolated Compounds

a) Compound 1

Compound 1 molecular formula was determined to be $C_{21}H_{19}O_{12}$ by HRTOFMS analysis and *m/z* of $[M-H]^+$ is 463.0880 (cal. 463.0877)

^1H NMR (300 MHz, MeOD-*d4*): δ 7.77 (1H, d, *J*=2.1 Hz), 7.62 (1H, dd, *J*= 8.5, 2.1 Hz), 6.89 (1H, d, *J*=8.6 Hz), 6.40 (1H, d, *J*=2.1 Hz), 6.23 (1H, d, *J*=2.1 Hz), 5.250 (d, 1H, *J* = 7.6 Hz). ^{13}C NMR (75 MHz, MeOD): δ 179.8 (C-4), 166.4 (C-7), 163.0 (C-5), 159.3 (C-2), 158.5 (C-9), 149.7 (C-3′), 145.9 (C-4′), 135.5 (C-3), 123.2 (C-6′), 123.3 (C-1′), 117.6 (C-2′), 116.1 (C-5′), 105.7 (C-10), 104.3 (C-1″), 99.9 (C-6), 94.7 (C-8), 78.4 (C-5″), 78.1 (C-3″), 75.9 (C-2″),71.1 (C-4″),62.7 (C-6″). The chemical structure was determined to be Quercetin glycoside

b) Compound 2

Compound 2 molecular formula was determined to be $C_{27}H_{30}O_{13}Na$ by TOF ES MS analysis and *m/z* of $[M+Na]^+$ is 649.1378.

^1H NMR (300 MHz, MeOD-*d4*): δH: 7.75 (1H, d, *J*=2.0), 7.69 (1H, dd, *J*=8.4, 2.0 Hz), 6.86 (1H, d, *J*=8.2 Hz), 6.44 (1H, d, *J*=1.9 Hz), 6.24 (1H, d, *J*=1.8 Hz), 5.26 (1H, d, *J*=7.3 Hz), 4.19 (1H, d, *J*=7.6 Hz). ^{13}C NMR (75 MHz, CD_3OD): δ 156.7 (C-2), 135.1 (C-3), 178.2 (C-4), 161.8 (C-5), 98.9 (C-6), 166.1 (C-7), 94.1 (C-8), 158.9 (C-9), 104.5 (C-10) 122.9 (C-1′), 115.3 (C-2′), 145.9 (C-3′), 146.5 (C-4′), 116.2 (C-5′), 104.6 (C-1″), 77.1 (C-5″), 76.9 (C-3″), 75.8 (C-2″),71.8 (C-4″),68.6 (C-6″), 103.3 (C-1‴), 81.5 (C-2‴), 76.8 (C-3‴), 71.5 (C-4‴), 73.8 (C-5‴), 62.2 (C-6‴). The chemical structure was determined to be Quercetin diglycoside

c) Compound 3

The molecular formula of compound 3 was determined to be $C_{36}H_{60}O_6$ by TOF ES MS analysis and *m/z* of $[2M]^+$ is 1176

^1H NMR (300MHz, CD_3OD); δH: 0.96 (3H, d, J=6.2Hz H-7), 0.99 (3H, d, J=6.4Hz, H- 5), 1.01 (3H, s H-14), 1.02 (3H, s, H-16), 1.21 (3H, s H-13), 1.43 (3H, s H-15), 2.63 (1H, d, 5=11.2Hz, H-18), 4.04 (1H, d, J=9.4 Hz, H-10), 4.23 (lH, d, J= 9.4, Hz, H-2) 5.47 (lH, s, H-12), 9.65 (lH, s, H-3), δH: 0.64 (3H, s, H-4), 0.65 (3H, s H-6), 0.78 (3H, s, H-9), 0.80 (3H, s, H-1), 0.82 (3H, d, *J*=1.67 Hz, H-11), 0.88 (3H, s, 19), 0.93 (3H, s, H-20) and 0.94 (3H, s, H-17). ^{13}C NMR (75 MHz, DMSO-*d6*): δ: 140.9 (C-13), 121.7 (C-12), 101.3 (C-1'), 77.3 (C-3), 77.2 (C-2'), 77.3 (C-4'), 73.8 (C-5'), 70.7 (C-3'), 61.6 (C-6'), 55.9 (C-5), 50.1 (C-

18), 49.1 (C-17), 45.6 (C-9), 42.3 (C-14), 40.2 (C-4), 39.7 (C-8), 39.4 (C-20), 39.2 (C-19), 38.8 (C-1), 36.9 (C-10), 35.9 (C-22), 31.9 (C-7), 29.9 (C-21), 29.1 (C-15), 25.9 (C-23), 25.9 (C-2), 23.1 (C-16), 23.1 (C-27), 21.1 (C-30), 19.6 (C-28), 19.6 (C-6), 19.4 (C-29), 19.1 (C- 26), 12.3 (C-24), 12.0 (C-25). The compound 3 was determined as Ursene-3-*O*-β-D-Glucopyranoside

B. Histological Slides Result

Photomicrographs (magnification X 160) shown in Appendix 17 are representative of hematoxylin and eosin stained slides of cardiac muscle of mice in all the treatment groups. In the slides of the groups administered with distill water, digoxin, crude extract, compound 1 and 2, there was normal arrangement of cardiac muscle tissue and presence of deeply staining basophilic nuclei, however, increased cellularity was also observed. In groups administered with compound 3, there is disorganization of cardiac muscle architecture and increased cellularity. No obvious sex differential effects were observed.

Photomicrographs (magnification X 160) shown in Appendix 18 of slides stained with Verhoeff's von geison stain showed normal cardiac architecture in groups given distilled water, digoxin, crude extract, compound 1 and 2, while representative slides of groups given compound 3 show dilatation of the cardiac muscle and some degenerative nuclei. No changes in elastic tissue was observed in any of the groups. Also no obvious sex differential effects were observed.

C. Discussions

Haematoxylin and Eosin staining technique provides an overview of the structure of the tissue which show the presence of basophilic nuclei promotes tissue repair [16].

In emphysema patients Verhoeff's Von Geison helps in the demonstration of the lungs with atrophy of elastic tissues and also in people with arteriosclerosis, and other vascular diseases, it helps in explanation of the thinning and loss of elastic fibers[17]. Elastic fibers are connective tissue fibers which enhances tissues to stretching, and which are found abundantly in the aorta, which aids flexibility to the large blood vessel [18]. This connective tissues are also present in other tissues that need elasticity, such as lung and skin. In haematoxylin and eosin stained tissue sections no elastic fibers was observed during the routine, therefore advance stains are required to highlight them.

From the Photomicrographs of hematoxylin and eosin and Verhoeff's Von Geison stain, digoxin, the crude extract, quercetin analogues (compound 1 and 2) have the same histological effect on the heart tissues (with preservation of the tissues of the heart) while the triterpene (compound 3) showed disorganization of the heart tissue. The cardiac glycosides (digoxin) has been reported to have prolonged depolarization of the cardiac cell [19] as shown in compounds 1 and 2, which is caused by increasing cellularity as shown in both hematoxylin and eosin and Verhoeff's Von Geison staining of the mice heart tissue. The triterpene (compound 3) was obtained in very minute quantity which may have no significant effect on the mice.

IV. CONCLUSION

It was found out that flavonoid glycosides (Quercetin glycoside and Quercetin diglycoside) present in *A. esculentus* preserved the cardiac muscle architecture similarly to digoxin the classical cardiac glycoside except Ursene-3-O-β-D-Glucopyranoside which shows dilatation of the cardiac muscle and some degenerative nuclei. Histologically, there is increases cellular infiltrates and hyalinization in all the test samples. The extract and compounds 1 and 2 have the same histological effect on the heart tissue which shows that the cardiovascular activities of *A. esculentus* is due to the presence of the flavonoid glycosides.

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