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CORTICAL SYNAPTOPHYSIN IMMUNOREACTIVITIES OF *CITRULLUS LANATUS* EXTRACT, SOYA BEAN OIL AND OMEGA H3

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ABSTRACT

The distribution of immuno reactivity of cortical synaptophysin was examined in the cerebrum of adult rats treated with seed extract of *Citrullus lanatus,* Omega H_3 and soybean oil. Twenty (20) adult wistar rats of average weights 150g were used. The rats were randomly divided into 5 groups of 4 rats per group. Group A served as control, groups B and C received 100mg/kg and 200mg/kg of *Citrullus lanatus* ethanolic seed extract respectively, while groups D and E received 300 mg/kg of Omega H3 and 5ml Soybean oil for two weeks respectively. Serum lipids were estimated biochemically and statistically analyzed, Result was presented asmean ± standard deviation, $P< 0.05$ was considered statistically significant. The cerebrum was dissected, fixed, processed and stained for synaptophysin. The result shows Soya bean oil significantly increased total serum cholesterol and serum Low Density Lipoprotein compared with the seed extracts of *C. lanatus* and omega H_3. Ethanolic seed extract of *C. lanatus,* Omega H_3 and Soya bean oil had no significant effect on serum triglyceride. The low dose of the seed extract and Soya bean oil significantly decreased High Density Lipoprotein. In conclusion, the seed extracts of *C. lanatus* shows dose dependant

enhancement activity on cortical synaptophysin than the other dietary supplements used in the study.

Keywords: Synaptophysin, *Citrulluslanatus*, Lipoproteins, Cholesterol, Omega H3, Soyabean

INTRODUCTION

Plants or herbs are used worldwide in traditional medicines for the treatment of various diseases [1]. The medicinal values of these plants lie in their phytochemical agents which are known to produce definite physiological effects on the body [2]. Plant seeds serve as sources of oil, nutriceuticals, supplements and raw materials for food processing companies. Diets rich in specific fatty acids provide cure for a number of health problems or diseases [3]. Unsaturated fatty acids have been suggested to possess health benefits which include the prevention of cancer, heart disease, hypertension and autoimmune disorders [2, 3]. Currently, consumer's growing interest in improving their dietary intake is a driving force on the search for novel seed oils having unique fatty acid profiles and other beneficial components, including phytosterols and natural antioxidants [4].

Water melon *(Citrullus lanatus*) is a member of the *Curcubitaceae* family [5]. The seed contain highly nutritive oil, protein, carbohydrate, lipids, antioxidant, dietary fibers, vitamins and minerals [6]. These classes of food found in watermelon seed may serve as sources of brain fuel particularly the lipids which form the major structural composition of the brain. It's useful in cognition and behavioural functions of the brain and as well enhances brain structural integrity. The major lipids found in the seed include: polyunsaturated fatty acids such a s linoleic acid, oleic acid, palmitic and stearic acid [7]. In our previous studies, *citrullus lanatus* extract exhibited enhanced haematopoietic activity of the bone marrow, immunoreactivity to the neurofilament in the hippocampus and total serum proteins in rats given same diets [8, 9]. These implicated *citrullus lanatus* seed extract in the enhancement of the structural integrity of the brain and boosting of bone marrow activity.

Similarly, Soybean oil is one of the edible seed oils with excellent fatty acid profile with relatively low saturated and high polyunsaturated fatty acids as well as monounsaturated fatty acids. It's also the principal source of omega-3 fatty acids and the primary commercial source of vitamin E in diet. The Omega-3 fatty acids (Omega-3s) are a group of polyunsaturated fatty acids prescribed medically as supplement to enhance brain function. The Omega-3 fatty acids are found in seafood's such as fatty fish and shellfish and vegetable seed oils and contain richer elements required

for the normal function of the body particularly the brain [10].

Polyunsaturated fatty acids (PUFA) play important roles in brain fatty acid composition and behaviour through their effects on neuronal properties and gene expression.[11] Changes in the PUFA content of the neural membrane influence membrane fluidity, control the physiological functions of the brain, and also regulate synthesis and functions of brain neurotransmitters resulting in changes in synaptic plasticity and spatial cognition [11, 12]. Fatty acids are crucial factors that determine the structure and function of biological membranes, including membranes in the nervous system [11, 13]. Studies have shown that PUFA supplementation is associated with an over-expression of synaptophysin in the hippocampus [14]. A sub-type of PUFA such as n-3 PUFA incorporated into the neuron membrane increase synaptic protein expression, neurite outgrowth, synaptogenesis, and neurogenesis to strengthen synaptic plasticity, protect the neurons and also influences the neural membrane biogenesis [11]. This present study evaluates effects of watermelon seed, Soya bean oil and Omega-H3 on lipid profile and their immunoreactivity on cortical synaptophysin.

MATERIALS AND METHODS

Preparation of ethanol extract of Citrullus lanatus seed

Fresh watermelon fruits (*C. lanatus*) were purchased from fruit sellers at Abakaliki Main Market, Abakaliki Ebonyi State. The fresh seeds of *C. lanatus* were air dried and selected to remove all seed with signs of disease, infection and insect bites. The seeds were pulverized using electric blender. 250g of the dry pulverized seeds was then submerged into 500ml of ethanol and stirred vigorously for 30-45 minutes. The mixture was filtered using a sieve over a funnel. Thereafter the residue was discarded while the filtrate was poured into an open tray and was exposed to air for 48 hours, to allow the evaporation of the alcohol. The extract was collected and stored in dark container until required for administration.

Drug and chemical

Commercially made soya bean oil (Grand pure soybean oil, Grand Cereals UAC Nigeria plc Jos. Nigeria) was purchased from Abakaliki main market. Omega H3 was purchased from a registered pharmacy store in Abakaliki Town.

Experimental Design

Healthy Wistar strain male and female albino rats of about two months old and weighing 150- 160 g were procured from the Animal House, Faculty of Basic Medicine, Ebonyi State University Abakaliki. The rats were housed in suitable

metal cages and maintained under environmentally controlled room provided with a 12:12 hour light and dark cycle approximately at 25°C ± 2. They were fed on pellets and tap water *ad libitum.* The rats were allowed to acclimatize to laboratory environment for two weeks.After the acclimatization period the rats were randomly assigned to five equal groups;Group A (control) was given 0.1ml normal saline (vehicle), Groups B received 100mg/kg ethanolic extract of watermelon seed, C received 200mg/kg ethanolic extract of watermelon seed and groups D received 300mg/kg Omega H3 and E received 5ml of Soyabean oil. All animals received extracts for 14 days.

Blood Collection

Twenty four hours after the last treatment blood was collected at 8:00 – 10:00 hours via orbital sinus using a capillary tube. The blood sample was collected in Non-EDTA bottles.

Serum lipid profile analysis

Determination total Cholesterol

The method of Uroko*et al.,* [15] was followed. Three (3) test tubes were set up in a test tube rack and labelled blank, standard and sample respectively. 10µl distilled water was added to the blank, 10ul standard specimen to the standard test tube and 10ul sample (serum) to the sample test tube. To each of these test tubes was added 1000ul of the cholesterol reagent. It was thoroughly mixed and incubated for 10minutes at room temperature (20-25^{o}C). The absorbance of the sample (A_{sample}) against the blank was taken within 60 minutes at 500nm.

Determination of Total Low Density Lipoprotein (LDL)

The serum samples were kept at 2-8^{0}C. The precipitant solution (0.1ml) was added to 0.2ml of the serum sample and mixed thoroughly and allowed to stand for 15 min. This was centrifuged at 2,000 x g for 15 min. The cholesterol concentration in the supernatant was determined. The concentration of the serum total cholesterol as described by Uroko*et al.,* [15]

LDL-C (mmol/L) = Total Cholesterol (mmol/L) – 1.5 x Supernatant Cholesterol (mmol/L).

Determination of Total High Density Lipoprotein (HDL)

The precipitant solution 0.1ml was added to 0.3ml of the serum sample and mixed thoroughly and allowed to stand for 15 min. This was centrifuge at 2,000 x g for 15 min. The cholesterol concentration in the supernatant was determined. Determination of the concentration of the serum total HDL as described by Uroko*et al.,* [15] was followed.

Determination of Total Triacylglycerol

Triacylglycerol (TAG) concentration was determined according to the method described by Uroko*et al.,* [15]. The triacylglycerol concentration is determined

by the enzymatic hydrolysis of triacylglycerol and a couple of reactions that generate quinoneimine as an indicator. The absorbance of the quinoneimine generated at 546 nm

Immunohistochemical study for synaptophysin

On day one post administration animals in all the groups were anaesthetized by 50mg/kg ketamine injection and the brain was carefully dissected out of the skull for each of the rats and immediately fixed in 10% formol saline for 48 hours. Thereafter the cerebrum of the rats were trimmed out using a surgical blade and fixed in 10% formol saline.

Fixed specimens were dehydrated in an ascending grade of alcohol, cleared in xylene and embedded in paraffin wax. The processed tissues were sectioned at 10 microns on the rotary microtome and placed on the hot plate at 70 degrees for at least 1hour.Sections were brought down to water by passing them on 2 changes of xylene, then 3 changes of descending grades (100%, 90%, 70% and 50%) of alcohol and finally to water. Antigen retrieval was performed on the sections by heating them on a citric acid solution of pH 6.0 using the microwave at power 100 for 15minutes.

The sections were equilibrated gradually with cool water to displace the hot citric acid for at least 5min for the section to cool. Peroxidase blocking was done on the sections by simply covering section with 3% hydrogen peroxide (H_2O_2) for 15minutes.Sections were washed with phosphate buffered saline (PBS) and protein blocking was performed using avidin for 15minutes.Sections were washed with PBS and endogenous biotin in tissues was blocked using biotin for 15minutes. After washing with PBS sections were incubated with the respective diluted primary antibody for synaptophysin antibody (manufactured by Novocastra, LEICA Germany) was diluted 1:100 for 60minutes.Excess antibodies were washed off with PBS and a secondary antibody (LINK) was applied on section for 15minutes.

Sections were washed and the (LABEL) which is the horseradish peroxidase (HRP) were applied on the sections for 15minutes. A working DAB solution is made up by mixing 1 drop (10microns) of the DAB chromogen to 1ml of the DAB substrate. This working solution was applied on sections after washing off the HRP with PBS for at least 5minutes. The brown reactions begin to appear at this moment especially for a positive target. Excess DAB solution and precipitate were washed off with water. Sections were counterstained with Haematoxylin solution for at least 2minutes and blued briefly. Sections were dehydrated in alcohol,

cleared in xylene and mounted in DPX. The slides were interpreted and photomicrographs were captured using Amscope research microscope model 3.2 at the Biotechnology center Ebonyi State University Abakaliki, Nigeria.

Statistical Analysis

The data were analysed the statistical package for social sciences (SPSS) version IBM computer USA 21. The results were presented in bar charts using mean ± standard deviation and at significant level of $P<0.05$.

RESULTS

Estimation of lipids

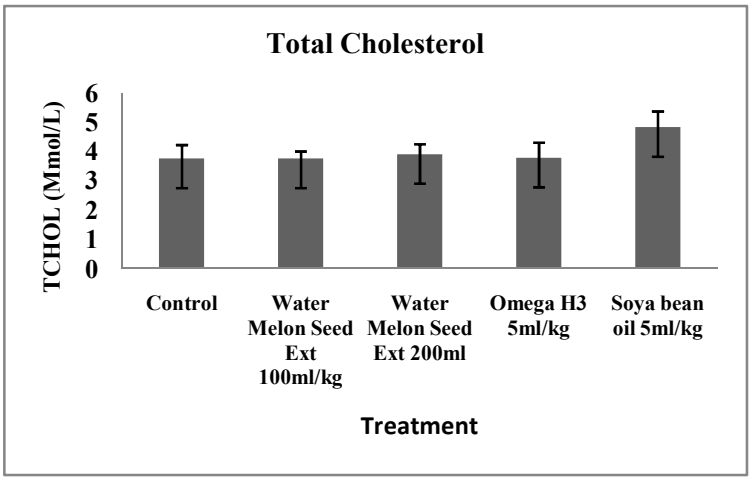

Figure 1: Bar chart representing the total cholesterol level of the control and treated rats at significant level of $P< 0.05$

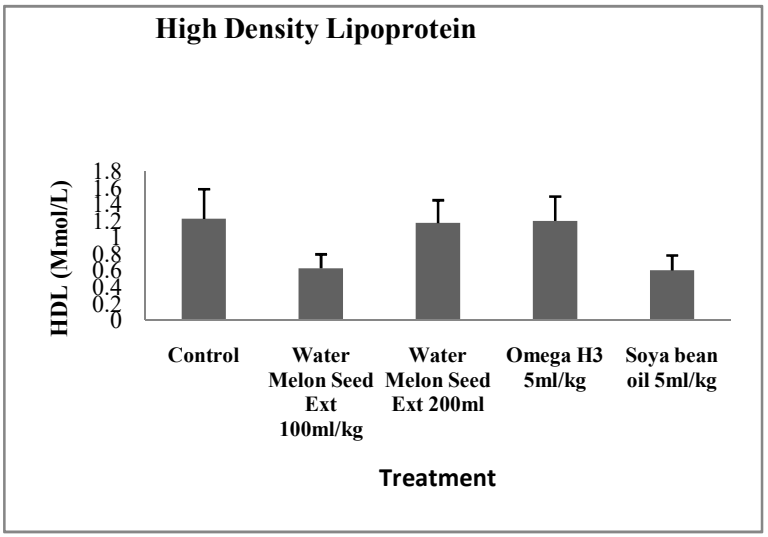

Figure 2: Bar chart showing High Density Lipoprotein of the control and treated rats at significant level of $P< 0.05$

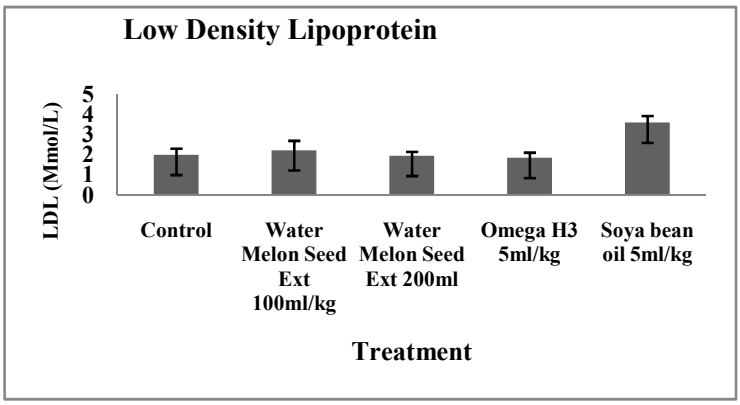

Figure 3: Bar chart showing low density lipoprotein level of control and treated rats at significant level of P< 0.05

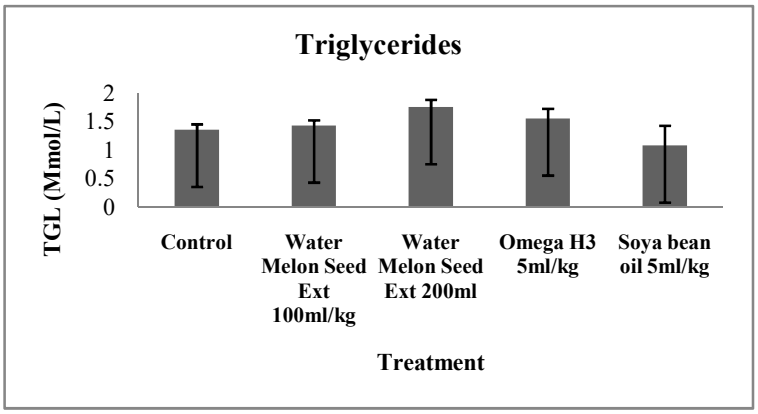

Figure 4: Bar chart showing triglycerides level of the control and treated rats at significant level of *P*<0.05.Administration of 200mg/kg of the water melon seed extracts exhibit higher triglyceride level

Immunohistochemical findings

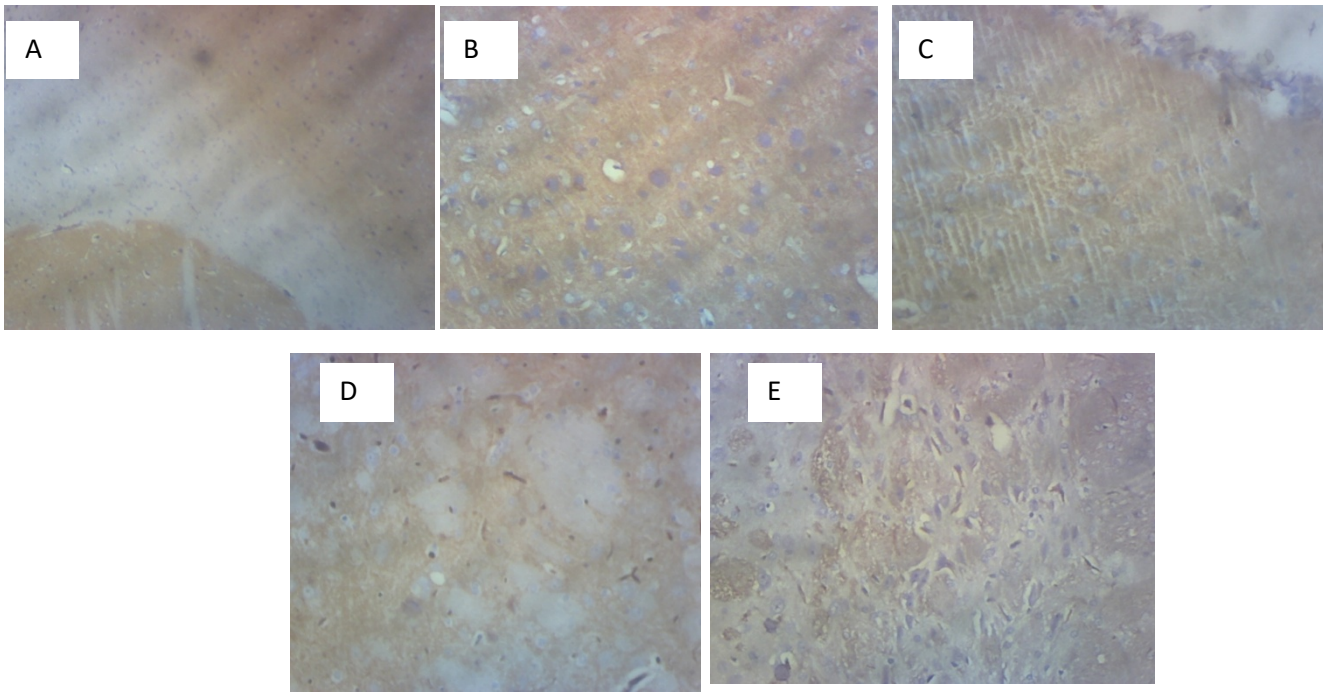

Figure 5: Photomicrograph showing sections of cerebrum of wistar rat of (A) control (B) 100mg/kg Extract (C) 200mg/kg Extract (D) Omega H_3 and (E) Soyabean oil. Increased synaptophysin reactivity in treated groupsB, C and D, and decreased in group E compared to the control that received 0.1ml saline. Synaptophysin .x200

DISCUSSIONS

Cholesterol is a steroid compound found in most body tissues, including the blood and nerves in the CNS. Cholesterol and its derivatives are also important constituents of cell membranes and precursors of other steroid compounds.[15]It is transported through the bloodstream by carriers called lipoproteins, which are so named because they are made of fat (lipid) and proteins. [16] The two types of lipoproteins that carry cholesterol to and from cells are low-density lipoprotein, (LDL) and high-density lipoprotein, (HDL). LDL cholesterol and HDL cholesterol, and one fifth of triglyceride level, constitute total cholesterol count.

The CNS contains approximately quarter of the unesterified cholesterol in the body.[16] The cholesterol in the CNS comes almost entirely from *in situ* synthesis, however, changes in cholesterol balance cause alterations in sterol recycling and expression of apolipoprotein E (ApoE) in the brain, leading to detrimental effects on neurons and glial cells. [16]This study shows a significant increase in the total serum cholesterol among all fed with soya bean oil. There was no significant change in serum constituent of groups fed with *Citrullus lanatus* seed extract and omega H3 when compared with the control group. These changes were within the permissible normal total serum cholesterol value (below 5.2mmol/l).

Cholesterol plays an important role in neuroarchitecture and the transmission of neural impulses. It is a key component of neuronal cell membranes, nerve growth cones, and myelin [17]. Cholesterol is not uniformly distributed in neuronal cell membranes, but is concentrated in micro domains, called lipid rafts. These heterogeneous areas in the membrane are enriched in gangliosides and thought to function as a platform for signalling pathways [18, 19]. Observational studies in humans have linked total serum cholesterol with measures of cognitive function, such as verbal fluency, attention, and abstract reasoning [20].

On the other hand, Low-density lipoprotein (LDL) is one of the five major groups of lipoprotein. The normal value for LDL is given at 2.59mmol/l, a border line of 3.37-4.12mmol/l and high at 4.15mmol/l and above. In this study the total serum LDL across the groups maintained a normal LDL value below 2.59mmol/l with the exception of the soya bean oil group with borderline value when compared to the control group. In addition, the 100mg/kg and 200mg/kg of *citrullus lanatus* maintained the normal value of LDL.

Unlike the LDL, the normal value of HDL cholesterol is given at 1.1-1.4mmol/l. It is shown from the result that the

administration of *citrullus lanatus* seed at 200 mg/kg and omega H3 at 5ml/kg increases HDL. This shows that effect of the seed extract is biphasic or dose dependent.Triglycerides are a common type of fat that accounts for about 95 per cent of all dietary fats found in both animal and vegetable fats. Once digested, triglycerides circulate in the bloodstream to be used as energy by the cells. Any leftovers are stored in body fat to fuel the body between meals. Thenormal value of triglyceride is below 1.7 mmol/L and high at between 2 and 6mmol/l. In this study, the values of triglyceride of the groups treated with dietary supplement when compared with the control group maintained normal triglyceride value that marked below 1.7mmol/l. It could be deduced from the result that administration of watermelon seed extract at 100mg/kg and 200mg/kg, Omega- H3 at 5ml/kg and Soya bean oil at 5ml/kg poses no danger of hypertriglyceridemia.

Synaptophysin is a major synaptic vesicle protein p38, encoded by the SYP gene [21]. It is present in neuroendocrine cells and in virtually all neurons in the brain and spinal cord that participate in synaptic transmission. [22] Its ubiquity at the synapse has led to the use of synaptophysin immunostaining for quantification of synapses [23]. The present study shows that synaptophysin is present in cerebral neurones and glia. The control group which received normal saline showed normal immunoreactivity to synaptophysin. In group B (100mg/kg seed extract), the reactivity to synaptophysin decreased, which implies decrease in synaptic vesicle activity.

Meanwhile group C (200mg/kg seed extract) increased synaptophysin reactivity. This implies there is increase in synaptic vesicle protein which can translate to increased synaptic transmission. In addition, extract showed dose dependent effect on synaptophysin reactivity. Also in group D (5ml/kg of omega H_3) there is clear increase in synaptophysin reactivity, which implies increase in synaptic vesicle protein and consequently increased synaptic activity. In group E (5ml/kg of soya bean oil), the reactivity to synaptophysin decreased which suggest decrease in synaptic vesicle protein. Put together the high dose of *C. lanatus* seed extract (200mg/kg) and Omega H_3 have synaptophysin enhancing effects than of the low dose *C. lanatus* seed extract (100mg/kg) and soya bean oil.

CONCLUSION

Soya bean oil significantly increased total serum cholesterol and serum Low Density Lipoprotein compared with the seed extracts of *C. lanatus* and omega H3. Ethanolic seed extract of *C. lanatus,* omega H3 and soya bean oil do not show

significant effect on serum triglyceride. The low dose seed extract and soya bean oil significantly decreased High Density Lipoprotein. The seed extracts of *C. Lanatus* shows dose dependant enhancement activity on cortical synaptophysin than the other dietary supplements used in the study.

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