Effects Of Ethanolic Extract of Aju Mbaise on High-Fat Diet induced Oxidative Stress, Body Weight, and Thyroid Gland histomorphology of Adult Wistar Rats

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Abstracts: Aju Mbaise is an ethnic Igbo herbal mixture known to contain a mixture of different plant parts. It is most popularly used by women for weight reduction. It contains different phytochemical compounds with antioxidants, antilipidemic, antiviral and antibiotic properties. High-fat diet (HFD) induces lipid peroxidation thus inducing oxidative stress in the body. The aim of this study was to investigate the effect of ethanolic extract of Aju Mbaise on the weight, oxidative stress markers (SOD, MDA, CAT, GPx) and the thyroid gland of albino rats fed with HFD. Thirty (30) adult female albino rats were put into 6 groups of 5 rats each. Group 1 (control group) received normal saline, group 2 was fed HFD for 21 days, then normal saline for 28days, group 3 received HFD for 21days, then 200mg/kg (low dose +) of Aju Mbaise for 28days, group 4 received HFD for 21days, then 400mg/kg (medium dose) of Aju Mbaise for 28days, group 5 received HFD for 21days, then 800mg/kg (medium dose) of Aju Mbaise for 28days. On day 50, blood samples were collected via retroorbital puncture in plain specimen bottles. The rats were then anaesthetized with 50 mg/kg thiopental sodium and aortic perfusion fixation with 4% paraformaldehyde was carried out. The thyroid glands were harvested, fixed in 10% buffered formalin and processed for routine H&E. The oxidative stress markers (SOD, GPx, CAT, and MDA), and body weight were evaluated. The results showed that Aju Mbaise reduces weight in a dose dependent manner. Group 2 (HFD only) showed reduction in SOD, CAT and GPx levels when compared with group 1 (control). SOD and CAT increased in group 6 (HFD + high dose Aju Mbaise) but GPx decreased. From the results, Aju Mbaise, at high dose, reduces body fat. The results also show that Aju Mbaise can reduce HFD induced oxidative stress and body weight in wistar rats. The histological evaluation of the thyroid gland showed changes in the follicular cells with graded doses of Aju Mbaise which is an indicator of increased thyroid function due to administration of Aju Mbaise.

Keywords: Oxidative Stress, Oxidative Stress Markers, Aju Mbaise, High-Fat Diet, Thyroid Gland

1. INTRODUCTION

Oxidation is a process necessary for the normal functioning of the biological systems in the body. Oxidative stress however, occurs due to the imbalance between the free radicals activity and antioxidant activity. To counter the harmful effect of oxidation, the body has a defense system known as antioxidant defense system comprising non enzymatic and enzymatic systems. Oxidative stress from a biological viewpoint has some positive benefits. For instance, oxidative stress induces apoptosis to ready the birth canal for delivery. Consequently, biological defense mechanisms are strengthened by oxidative stress during the appropriate physical activities and ischemia. Although most free radicals result due to oxygen metabolism, there are some instances of free radicals exposure in the environment. These sources includes: radiation, and environmental pollutionvia certain pesticides and cleaners, cigarette smoke,etc. Food diet containing large amount of sugar, fat and alcohol may also contribute to free radical production [1]. With these, we can clearly define oxidative stress as defined by Yoshikawa and Naito as a state where oxidation exceeds the antioxidant systems because of the loss of balance between them [2].

The body has its own defense mechanism against the reactive oxygen species (ROS) called the antioxidants; they tend to scavenge the free radicals thereby reducing oxidative stress. The antioxidants found in human body are classified as small molecules, enzymes and proteins and are used as oxidative stress markers. However, antioxidants can also be found in food diets especially in fruits, vegetables and other plant-based whole foods. General types of antioxidative substances according to Naito *et al* are divided into water soluble substances such as Vitamin C, and fat soluble substances such as Vitamin A and E as well as coenzyme Q10 [3]. The antioxidants

mutually react with each other and separately to form protective networks around biological systems or membranes against oxidative damage.

Aju Mbaise is a traditional herbal therapy made with the combination of several leaves, roots and trunk of different medicinal plants all wrapped together. The herb has its origin from 'Mbaise' in Imo state, Nigeria. It is made up of *Sphenocentrum jollyanum, Cnestis ferruginea, Xylopia aethiopica, Uvaria chamae, Palisota hirsute, Scleria sp., Napoleona Sp., Dialium guineense, Combretum racemosun, and Heterotis rotundifolia.* [4].

According to WHO (as cited in Ekor,), many people resort to the use of herbal medicines and phytonutrients or nutraceuticals for the treatment of various health challenges in healthcare setting [5]. Aju Mbaise has been noted to contain an appreciable amount of metabolites which include alkaloids, tannins, flavonoids, cyanogenic glycoside and saponin likewise some mineral elements in the decoction which includes high levels of potassium, calcium, magnesium and sodium with low levels of iron, zinc, phosphorus, copper, manganese and chromium. [6]. Some of the therapeutic effects of Aju Mbaise include aphrodisiac, restorative activities which includes anti-diabetic, anti-oxidant, anti-inflammatory, anti-allergic, anti-bacterial, anti-malaria, and neuroprotective [4,7,8].

Thyroid gland, an organ located in the base of the neck, is part of the endocrine system that produces hormones regulating metabolism. The thyroid gland uses iodine from food diet to make two main hormones; the Triiodothyronine (T_3) and Thyroxine (T_4). The thyroid hormones regulate some vital body functioning such as the heart rate, central nervous system, peripheral nervous system, breathing, cholesterol levels etc [9].There is a complex relationship between thyroid hormone levels and oxidative stress. Due to the capacity of thyroid hormone to accelerate the basal metabolism, cellular reactions and changing respiratory rate in mitochondria, it plays a significant role in oxidative stress[10].

MATERIALS AND METHODS

Preparation of the Lard

Methods: Fat tissue was cut from the fat deposits in the pig abdominal walls, cut into pieces, washed thoroughly in running water and put inside a cooking pot. Water was added and boiled under constant temperature for 30 minutes. It was allowed to cool at room temperature, and the oil layer was sieved using a regular sieve cloth, poured it in a plastic container, and stored it in a refrigerator overnight.

Result: The content after refrigeration, gave a white semi-solid organic substance known as Lard very rich in lipids (fats).

2.3: **High Fat-diet preparation**

High fat diet (HFD) was prepared by mixing the prepared lard with sucrose and normal animal feed (broiler finisher pellets feed). The composition of the high-diet was calculated in the ratio of 3:1:1 i.e. animal feed, Lard, and sucrose respectively, and was carefully homogenized according to Nnadiukwu et al [4].

2.4: **Collection of Plant Samples**

Fresh samples of the plants that make up Aju Mbaise were procured from the dealers at Afor-Enyiogugu market in Amuzi, town in Aboh Mbaise L.G.A., of Imo State, Nigeria.

2.5: **Extraction of Herbal materials**

The plant materials were washed, air-dried for 72hours, weighed, and blended to powder. Powdered sample weighing 330g was soaked in 3,000ml of 95% ethanol for 72hours after which it was sieved using a muslin cloth and afterward filtered through a Whatmann filter paper No. 1. The filtrate was concentrated using a rotary

evaporator at 45 ^{o}C and afterward placed on a thermostatic water bath for further drying. The concentrate was collected, weighed, kept in a sterile bottle, and stored in a refrigerator at 4 ^{o}C until usage..

Experimental Animals and Design

Thirty (30) adult female wistar rats were procured from the Department of Pharmacology and Therapeutics, University of Nigeria Enugu Campus (UNEC).They were acclimatized to the conditions of the animal house of the Department of Anatomy, UNEC for a period of 7 days in iron mesh cages. All animals were provided with food and water *ad libitum* throughout the acclimatization period. Randomized controlled experimental design was employed on animal model. Experiments were carried out according to the guidelines for care and use of experimental animals. After 10 days of adaptation to the environment, the rats were randomly divided into 6 groups of 5 rats each and the ethanolic extract of Aju Mbaise was administered orally using orogastric tube. The experiment lasted for seven weeks. The treatment was as follows:

Group 1: received normal diet throughout the experiment.

Group 2: received only 400mg/kg body weight of Aju Mbaise for 49days

Group 3: received only high-fat diet for 49 days

Group 4: received high-fat diet for 21days, then 200mg/kg body weight of Aju Mbaise for 28days.

Group 5: received high-fat diet for 21days, then 400mg/kg body weight of Aju Mbaise for 28days.

Group 6: received high-fat diet for 21days, then 800mg/kg body weight of Aju Mbaise for 28days.

Table 1: Animal grouping for adult female Wister rats

Groups (n=5)	High fat diet (HFD)	Treatment	Dosage/kg body weight
Group 1	No HFD	Normal saline	2ml
Group 2	No HFD	Aju mbaise ethanolic extract	400mg (medium dose)
Group 3	HFD present	Normal saline	2ml
Group 4	HFD present	Aju mbaise ethanolic extract	200mg (low dose)
Group 5	HFD present	Aju mbaise ethanolic extract	400mg (medium dose)
Group 6	HFD present	Aju mbaise ethanolic extract	800mg (high dose)

Collection and analysis of samples

On day 50 of the experiment, the blood samples were collected from the rats in the retro-orbital venous plexus via the medial canthus and stored plain bottles for biochemical analysis. The rats were then anaesthetized with 50 mg/kg thiopental sodium and aortic perfusion fixation with 4% paraformaldehyde was carried out. The neck regions dissected to harvest the thyroid glands which were fixed in 10% formol saline. The tissues were then processed for routine H and E stains while the blood samples collected were used to analyze for oxidative stress parameters using ELISA kits. The oxidative stress markers that were analyzed includes: Superoxide dismutase (SOD), Malondialdehyde (MDA), Catalase(CAT), and Gluthatione Peroxidase (GPx). All procedures were carried out according to manufacturer's protocols.

Statistical Analysis

Data was analyzed using statistical package for social sciences (SPSS) version 23 (IBM computer USA). Data was presented as mean ±standard deviation. One way Analysis of variance (ANOVA) was used to compare the means. Difference between the means was considered significant at $p<0.05$.

Ethical Clearance

The ethical clearance was obtained from the research ethics committee of the Faculty of Basic Medical Sciences University of Nigeria, Enugu Campus with protocol No. UN/FBMS/HAREC/22/S.045.

RESULTS

Oxidative Stress Markers

Superoxide dismutase (SOD)

Superoxide dismutase level was significantly reduced in the group 3 (Fat diet only) compared to group 1(negative control) (figure 1). ($p<0.05$, ANOVA)

SOD level was significantly increased in the group 6 (Fat diet plus high dose AJB) compared to group 3(Fat diet only). ($p<0.05$, ANOVA)

The SOD level was comparable in group 1(Negative control) and group 6 (Fat diet plus high dose AJB).

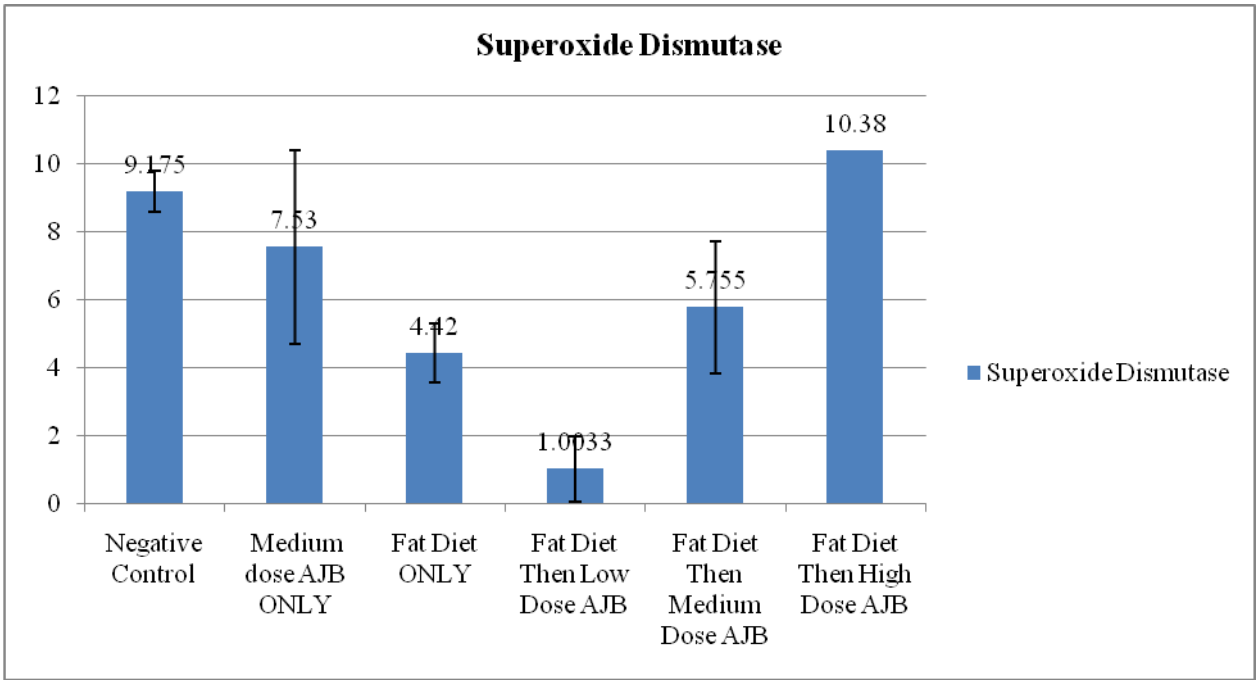

Fig.1: Superoxide Dismutase

Malondialdehyde

Malondialdehyde level was significantly increased in group 3(Fat diet only) and group 4 (Fat diet plus low dose AJB) compared to group 1(negative control). ($p<0.05$, ANOVA)

There was no significant difference in the mean Malondialdehyde level of group 5 (Fat diet with medium dose AJB) and group 6 (high dose AJB) compared to group 1(negative control). ($p<0.05$, ANOVA)

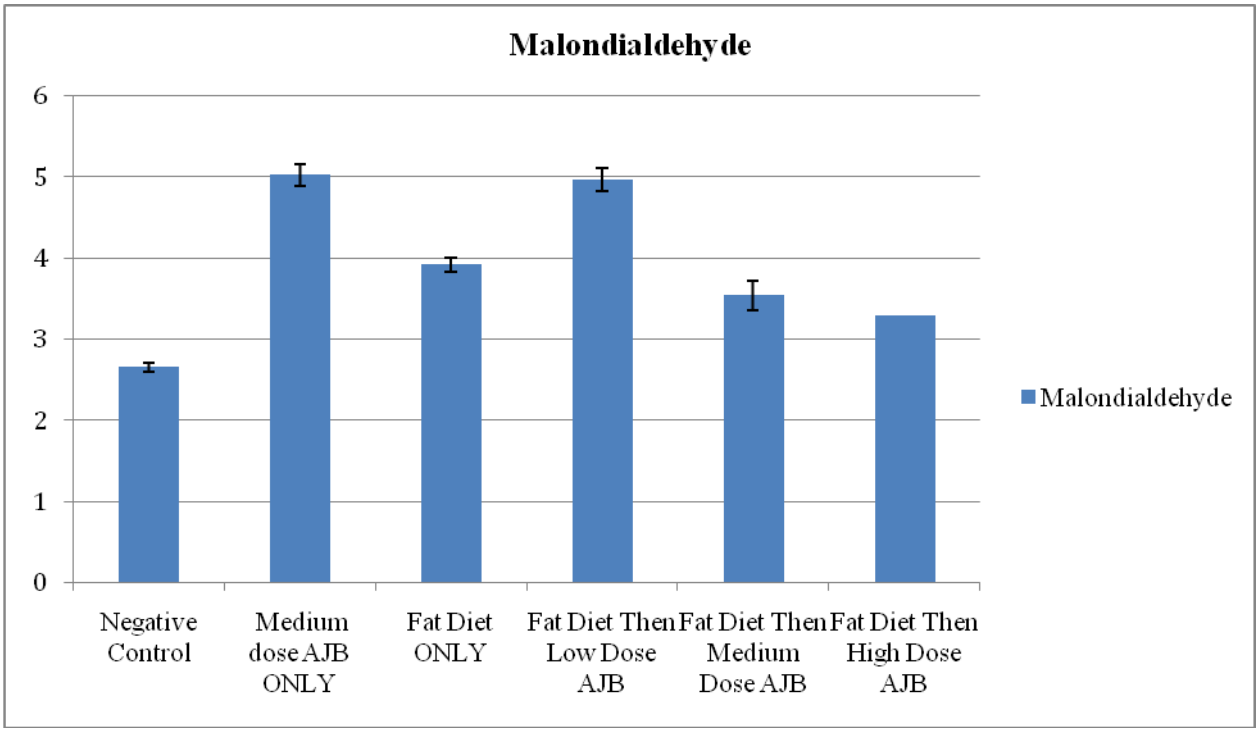

Fig.2: Malondialdehyde

Catalase (CAT)

Catalase level was significantly reduced in the group 3 (Fat diet only) compared to group 1(negative control). ($p<0.05$, ANOVA)

CAT level was significantly increased in the group 6(Fat diet plus high dose AJB) compared to group 3 (Fat diet only group). ($p<0.05$, ANOVA)

The CAT level was comparable in group 1(Negative control) and group 6(Fat diet plus high dose AJB).

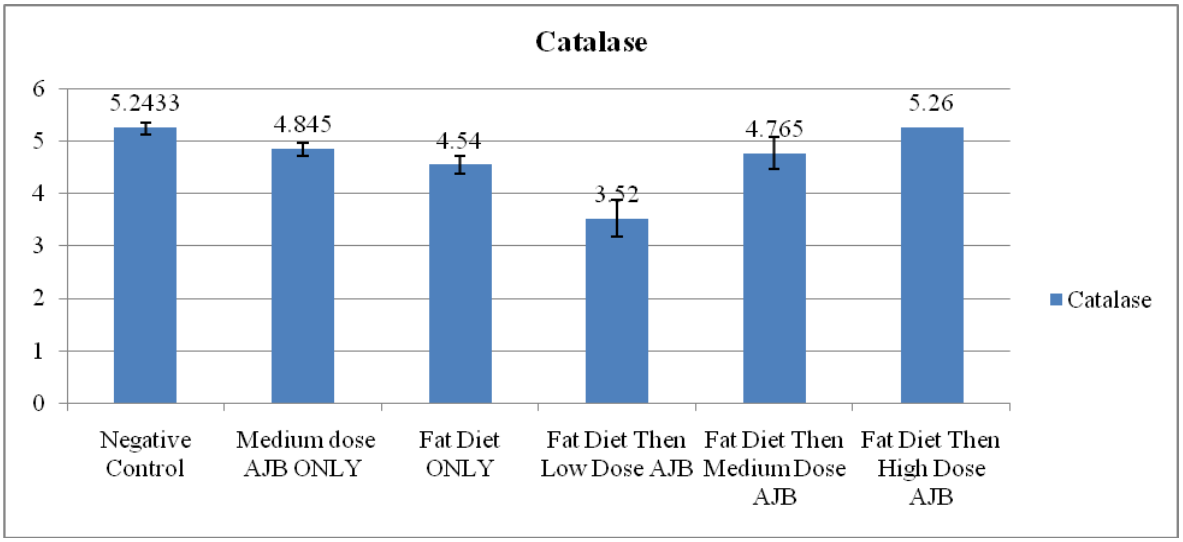

Fig.3: Catalase

Glutathione Peroxidase (GPx)

Glutathione Peroxidase level was significantly reduced in group 3 (Fat diet only) compared to group 1 (negative control). ($p<0.05$, ANOVA)

GPx level was significantly reduced in groups 2, 4,5& 6(AJB groups) compared to group 1(negative control). ($p<0.05$, ANOVA)

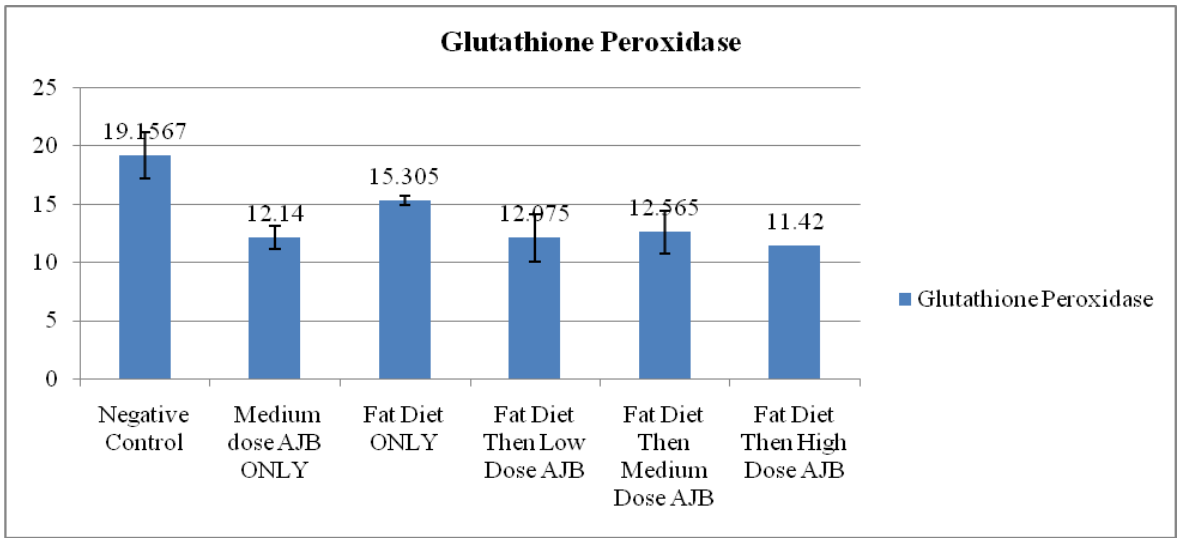

Fig.4: Glutathione Peroxidase

Body Weight

The Weight of the rats in group 3(high fat diet only) significantly increased at week one to six compared to group 1(Negative control).

High dose AJB significantly reduced weight in group 6(Fat diet plus AJB high dose) compared to group 1(Negative control).

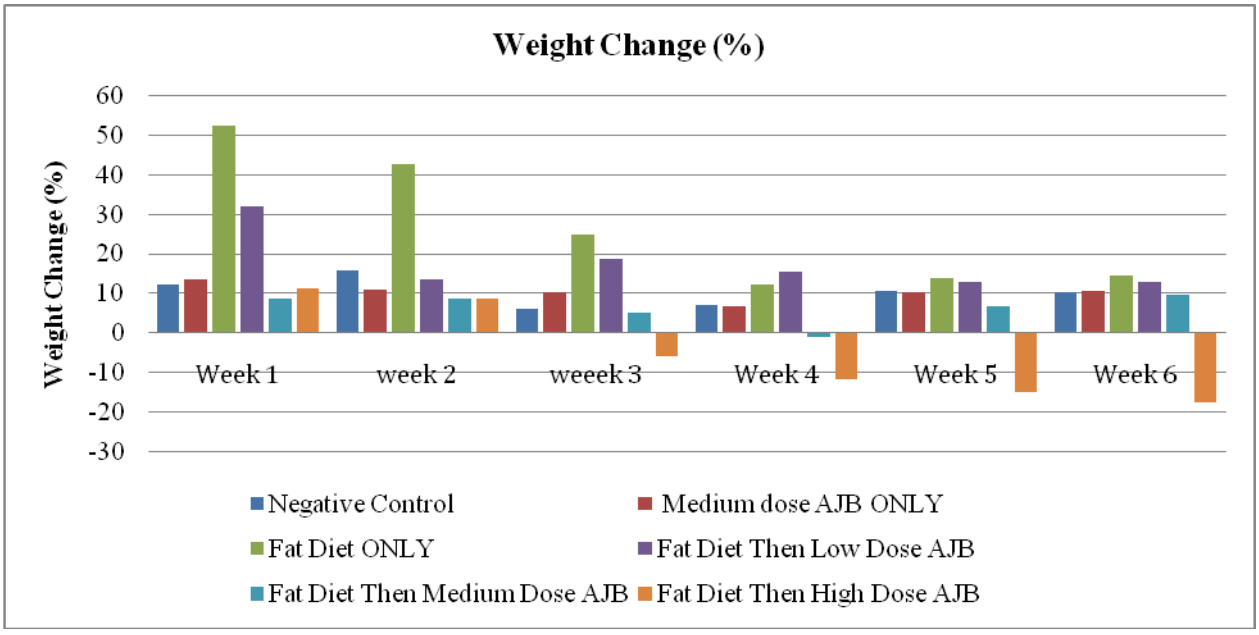

Fig.5: Weight Change (%)

Histological parameters for thyroid gland

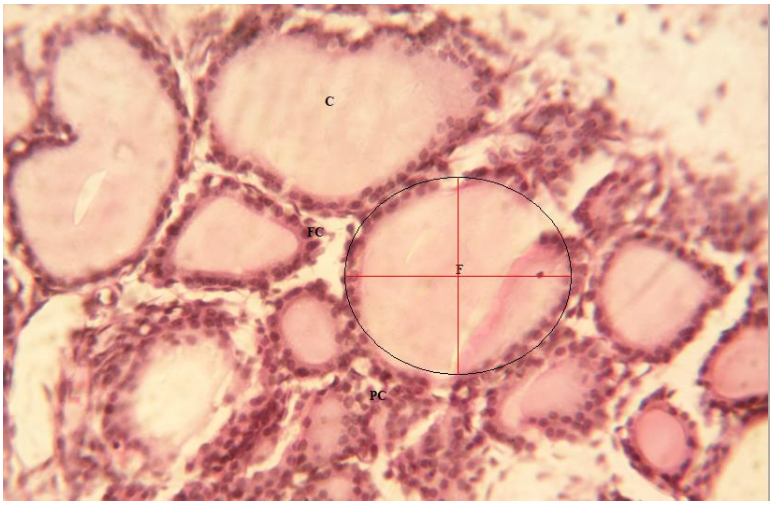

Plate 1: Photomicrograph of the thyroid gland of group 1(Negative control) showing colloid (C), thyroid follicle (F), follicular cells (FC) and parafollicular cells (PC). The follicular cells appearing cuboidal with a moderate amount of colloid H&E.x400.

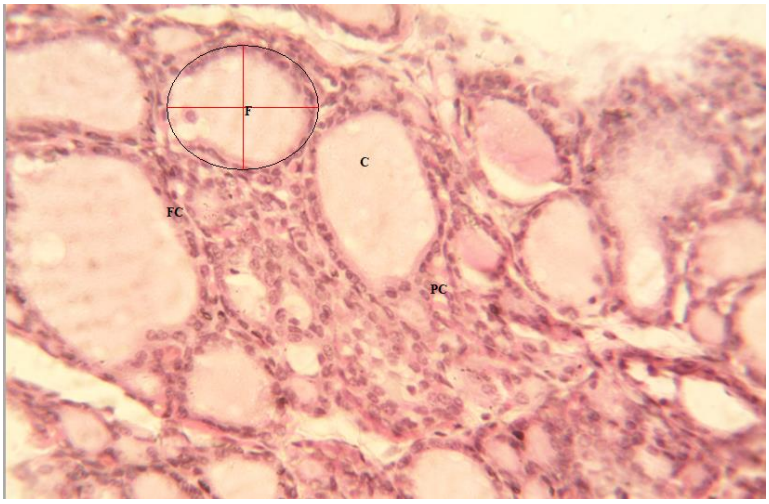

Plate 2: **Photomicrograph of the thyroid gland of group 2 (Medium Dose AJB Only) showing colloid (C), thyroid follicle (F), follicular cells (FC) and parafollicular cells (PC). Shows columnar follicular cells with diminished colloid H&E.x400**

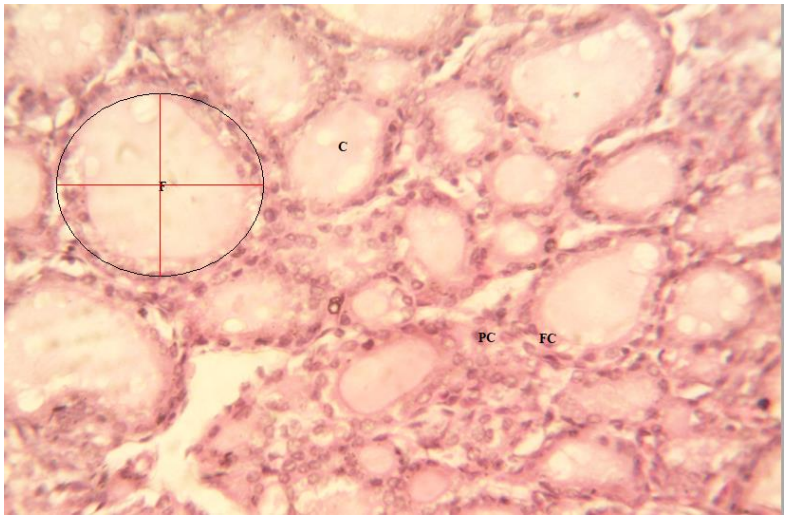

Plate 3: **Photomicrograph of the thyroid gland of group 3 (Fat Diet Only) showing colloid (C), thyroid follicle (F), follicular cells (FC) and parafollicular cells (PC). Shows cuboidal follicular cells, a decreased and heavily vacuolized colloid. H&E.x400**

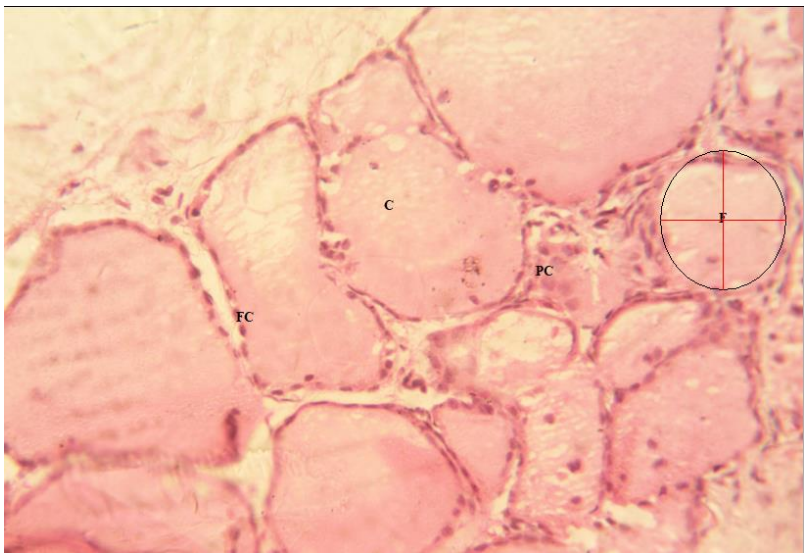

Plate 4: Photomicrograph of the thyroid gland of group 4 (Fat Diet Then Low Dose AJB) showing colloid (C), thyroid follicle (F), follicular cells (FC) and parafollicular cells (PC). Shows comparatively flattened follicular cells with moderate amount of colloid. H&E.x400

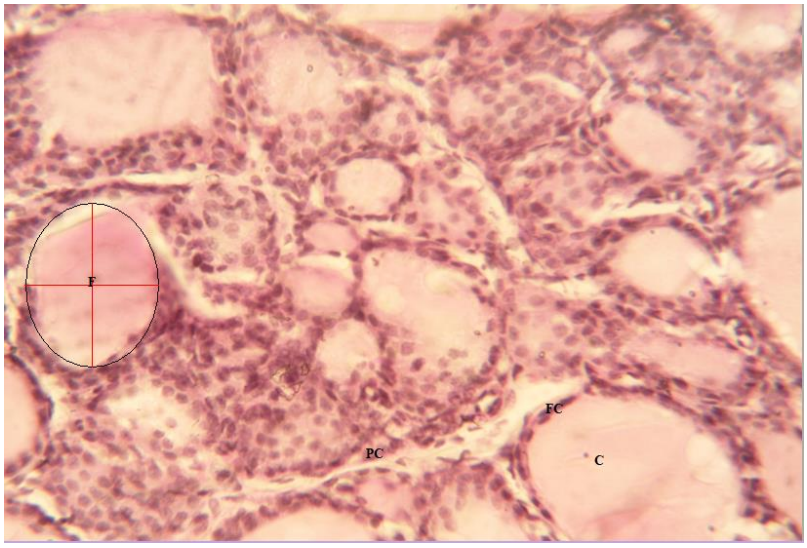

Plate 5: Photomicrograph of the thyroid gland of group 5 (Fat Diet Then Medium Dose AJB) showing colloid (C), thyroid follicle (F), follicular cells (FC) and parafollicular cells (PC). Shows the follicular cells to be cuboidal with slight vacuolization. H&E.x400

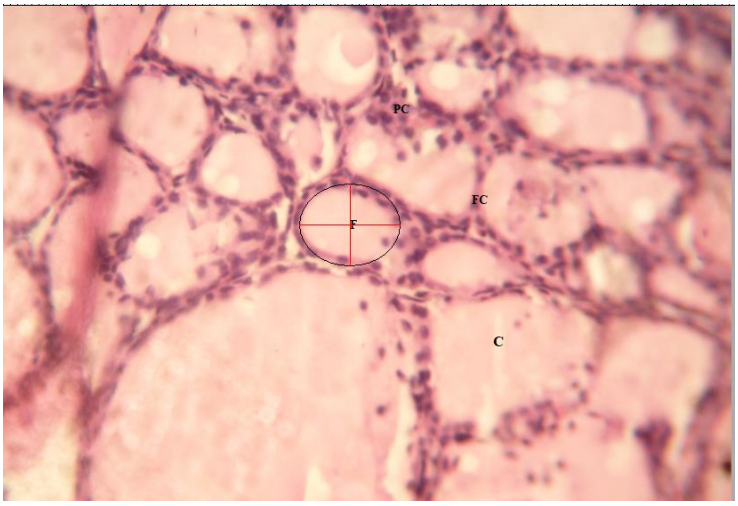

Plate 6: Photomicrograph of the thyroid gland of group 6 (Fat Diet Then High Dose AJB) showing colloid (C), thyroid follicle (F), follicular cells (FC) and parafollicular cells (PC). H&E.X400

DISCUSSION

Oxidative stress has been noted to occur due to the imbalance between the free radical activity and the antioxidant activity. Oxidative stress occurs when the oxygen free radicals attacks molecules in biological membranes and tissues such as lipids, proteins and DNA thus leading to various lifetime diseases which includes diabetes, hypertension, cancer, neurodegenerative diseases as well as aging [1].

It has been shown that SOD and CAT levels decrease when there is oxidative stress [11]. In this study, the decrease in SOD and CAT levels was statistically significant in group 3(fat diet only) when compared to group 1 (negative control) as shown in figures 4.1 and 4.3). This decrease in SOD and CAT levels suggests increased oxidative stress which may have resulted from increased ROS levels.

SOD and CAT was significantly increased in group 6(fat diet plus high dose AJB) when compared to group 3(fat diet only). This suggests that AJB had an effect on the increased level of SOD as it has been noted to contain antioxidants[4].

The SOD and CAT level was comparable in group 1(negative control) and group 6(fat diet plus high dose AJB).

MDA level was significantly increased in group 3(fat diet only) and group 4 (fat diet plus low dose AJB) when compared to group 1 (negative control). (Figure 4.2) This decrease in MDA level suggests an increased lipid peroxidation in the groups which may be due to increased ROS. MDA is a toxic product from lipid peroxidation of polyunsaturated fatty acids and is a well-known marker of oxidative stress [12].

There was no significant difference in the mean MDA level of group 5 (fat diet with medium) and group 6 (fat diet with high dose AJB) when compared to group 1 (negative control). This suggests increase in MDA level is not dose dependent of AJB.

GPx level was significantly reduced in group 3(fat diet only group) and groups 2,4,5,6(AJB groups) when compared to group 1 (negative control). This decrease in GPx suggests an increase in ROS which is a consequence of oxidative stress. Individuals with lower GPx activity are predisposed to impaired antioxidant protection which leads to oxidative damage to membrane fatty acids and functional proteins, which can be inferred to cause neurotoxic damage [13].

Nnadiukwu *et al,* noted the various phytochemicals of AJB to include alkaloids, flavonoids, glycosides, hydrogen cyanide, phenols, saponins, steroids, tannins and terpenoids[4]. Saponins are reported to exhibit cholesterol-

lowering action in animals and humans[4]. The weight of the rats in the high fat diet group significantly increased at week one to six compared to the negative control. This suggests that high fat diet increases body weight.

High dose AJB significantly reduced weight in the fat diet plus AJB high dose group compared to negative control. This suggests that AJB reduces weight at high dose and is also dose dependent.

The shapes of the follicular cells vary depending on their level of activity. They appear cuboidal on a normal level with a moderate amount of colloid. When inactive the cells are flat/squamous and the follicles are distended with abundant colloid. When the follicular cells are highly active, they become columnar and the colloid is scanty[14].

In this study, the thyroid gland of the negative control appears normal with the follicular cells appearing cuboidal with a moderate amount of colloid within the follicles.

When compared to the negative control, (medium dose AJB only), the thyroid histomorphology appeared to have columnar follicular cells with diminished colloid. This suggests increased thyroid activity.

The histomorphology of the thyroid gland of group 3 (HFD only) when compared to the negative control showed a cuboidal follicular cells, a decreased and heavily vacuolized colloid. The appearance of colloid vacoules is considered to be a typical sign of hyperactivity in the human thyroid gland[14].

The histomorphology of the thyroid gland of group 4 (HFD and low dosage AJB) when compared to the negative control showed a flattened follicular cells with moderate amount of colloid. This suggests a reduced thyroid activity.

The histomorphology of the thyroid gland of the high fat diet then medium dose AJB when compared to the negative control showed the follicular cells to be cuboidal with slight vacuolization. This suggests increased thyroid activity.

CONCLUSION

The results of this work suggests that Aju Mbaise reduces body weight, increases antioxidant levels thereby reducing oxidative stress and also leads to increased metabolic rate as shown in the increase in thyroid activity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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