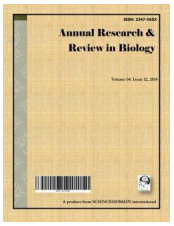

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# Comparative Effects of Methanolic Leaf Extracts of *Azadirachta indica* and *Spondias mombin* on the Hypothalamic-pituitary-adrenal Axis of Zidovudine Stress Induced Wistar Rats

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors COU and ORA designed the study, performed the statistical analysis and wrote the protocol. Authors COU and GEO wrote the first draft of the manuscript. Authors MAE and COU managed the analyses of the study. Authors COU, ORA and GEO managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

The hypothalamic-pituitary-adrenal axis functions in the maintenance of homeostasis in the various systems of the body. This study was carried out to evaluate the anti-oxidant effect of *Azadirachta indica* and *Spondias mombin on the* Hypothalamic-Pituitary- Adrenal axis of zidovudine stress induced wistar rats. 25 adult male wistar rats having an average weight of 180g were used for this study and were divided into 5 groups; group A, group B, group C, group D and group E. Group A is the negative control group that received rat chow and water , group B served as the positive control group that received the administration of 450mg/kg body weight of zidovudine drug, group C received 450mg/kg body weight of zidovudine drug and 500mg/kg body weight of *Azadirachta indica*, group D received 450mg/kg body weight of zidovudine and 500mg/kg body weight of *Spondias mombin* leaf extract, while group E received 450mg/kg body weight of zidovudine drug

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and a combination of 500mg/kg body weight of *Azadirachta indica and Spondias mombin.* The administration was carried out once a day using orogastric tube for a period of 21 days. At the end of the administration, the rats were sacrificed using chlorofoam inhalation technique, and the whole brain was fixed in 10% neutral buffered formal saline, Blood samples for biochemical estimation were taken. Light microscopic evaluation of the Hypothalamic-Pituitary-Adrenal axis using Haematoxylin and Eosin for group A showed prominent hypothalamic neurons, group B showed degeneration of neurons and presence of vacoules, group C, D,and E showed less vacoules and prominent neurons with lesser vacoules and more prominence of neurons in group C. Haematoxylin and eosin stains for group A, C, and E of the rat pituitary gland, showed prominent acidophilic and basophilic cells with presence of blood vessels, while results of group B and D showed presence of vacoulation with less prominent acidophilic and basophilic cells, Haematoxylin and eosin results for group A showed presence of all the 3 layers of the adrenal cortex and medulla, group B of rat adrenal gland showed presence of haemorrhage in the medulla layer and reticularis, group C had lesser haemorrhage when compared to group D and E. Results of Orange G showed prominence in the acidophilic and basophilic cells in Group A, C, D and E and B showed lesser prominence of orange G stains. Cresoyl Fast Violet stains showed more prominent nissl substance in group A, D, when and E while group B showed a reduction in the expression of nissl stains. Results for stastistical analysis of glutathione peroxidase showed an increase in GPX for group D, when compared to groupA, C and E, and B at ($p<0.05$), results of Malondialdehyde (MDA) significantly increased in group B ($p<0.05$) when compared to group A, C, D and E. Hence this study proves that single administration of methanolic leaf extract of *Azadirachta indica* or *Spondias mombin* may have neuroprotective potentials and anti-oxidant properties when compared with a combination of the methanolic leaf extract of *Azadirachta indica* and *Spondias mombin* leaf extract.

*Keywords: Adrenal gland; combination; hypothalamus; neuroprotection; pituitary gland.*

## 1. INTRODUCTION

Herbal products are of interest to many health care practitioners since about 70% of the worldwide population rely on herbal medicine for part of their primary health care [1]. Various sections and traditions make use of native substances as lone herbs, join of plants and drugs and union of herbs. When herbs are used in combination it could lead to complications as numerous association can happen within the person constituent. Complications may arise because of numerous constituent in the native extracts [1].

However the impacts from plant-plant association are likely uncertain and complex [2,3,4,5,6,7,8,9,10].

Oxidative stress can be defined as a disproportion among the system display of active kind air and a functional body capacity to remove the active intermediate or to restore the outcome injury [11]. It is caused when the existence of liberal substance overwhelms the free scavenging mechanism of anti-oxidants [12]. Oxidative stress can be caused by a number of factors; these include and not limited to; intake of processed foods, high fatty foods consumption, consumption of sugary foods, smoking of cigarette, marijuana, increase in alcohol consumption, as well as exposure to emotional and mental stress [13,14,15,16,17]. Oxidative stress could be managed by the intake of foods containing anti-oxidants such as Vitamins (A, C, E), fruits, vegetables, nuts, poliphenols, catechins so as to increase the level of anti-oxidants which has helped in fighting free radicals [18].

*Azadirachta indica* (neem tree) is a native plant of South eastern Asia, and it is distributed in India and other neighboring countries [19]. It is called dogonyaro in Hausa, and Ogwuakuma in Igbo [20]. *Azadirachta indica* plays therapeutic role in the management of health due to the presence of rich source of various types of ingredients. Most important active chemical components of *Azadirachta indica* is Azadirachtin, nimbolin, nimbin, nimbol, sodium nimbinate, gedunin, salannin and quercetin [21]. *Azadirachta indica* is rich in phytochemical constituents like azadiratchin, nimbolide and ascorbate which possess significant anti-oxidant properties, that enables it to scavenge free radicals present in the body [22].

*Spondias mombin* belongs to the family *Anarcadiaceae*, and it is one of the medicinal herbs in southern Nigeria [23]. It has several

names; it is termed English in plum hog, Yoruba akika, tsardamaster in Hausa, Chabbuh in Fulani and nuskakara in Efik [24]. *Spondias* also possess anthelminthic, anti-oxidant, anti-microbial and anti-inflammatory actions, sedative and anxiolytic potentials [25,26,27,28,29,30].

The Hypothalamic-Pituitary-Adrenal axis (HPA) is a neuroendocrine system made up of three endocrine glands; the hypothlamus, Pituitary, and gland Adrenal. This axis performs negative feedback interactions amongst the three endocrine glands. This axis functions in controlling reactions to stress, control of many system activities such as intake, emotion, sex feeling, power reservoir and wastage. It also functions in the regulation of homeostasis of some systems in the body which are heart channel, protected, system nervous central and system repllicative [31]. Therefore this study was carried to evaluate the effects of oxidative stress on the HPA axis and also to compare the impacts of single administration of herbal extracts with the combination of herbal extracts in ameliorating the effects of oxidative stress.

## 2. MATERIALS AND METHODS

The leaves *Azadirachta indica* and *Spondias mombin* were obtained from a local community in Ugep, Yakurr local Government Area of Cross River State, Nigeria. Taxonomical identification was conducted by a botanist in the Department of Botany University of Calabar, Calabar, Nigeria. With a voucher specimen already existing. Both leaves were powdered and extracted with by cold extraction method using methanol as the solvent for a period of 72 hours with the aid of a soxhlet apparatus. The extract were filtered through whatmann paper 1 and the filtrate was evaporated to dryness on rotary evaporator at (50℃). The extract were preserved in clean glass container for further use.

### 2.1 Animals

This study was approved by the Department Ethics Committee of the\University of Calabar, Calabar. Twenty-five male adult Wister Rats with an average weight of 200 g were bred in the animal house of the department of Anatomical Sciences and were used for this study. The rats were fed with rat chow, water ad libitum.

### 2.2 Experimental Protocol

This study was carried out using twenty-five male adult wistar rats “average of 200g “and there were randomly distributed into five sections (A, D,E, B,C, n=5).

Group A the Negative normal group that distilled water and rat chur, Group B is the Positive control group that was induced with 450mg/kg body weight of zidovudine drug for a period of three weeks. Group Cis the Experimental group that was induced with 450mg/kg body weight of zidovudine drug for a period of one week and received 500mg/kg body weight of *Azadirachta indica* for a period of two weeks. Group D represents Experimental group that was induced with 450mg/kg body weight of zidovudine drug for a period of one week and received 500mg/kg body weight of *Spondias mombin* for a period of two weeks. While Group E Experimental group received 450mg/kg body weight of zidovudine drug for one week and 500mg/kg body weight of *Azadirachta indica* and *Spondias mombin* for a period of two weeks. At the end of the administration, the animals were anaesthesised using chlorofoam inhalation technique.

### 2.3 Stress Induction

Oxidative stress was induced using Zidovudine obtained from the Plan President Emergency for Aids and liberation section, Teaching University of Calabar Hospital, Calabar town, Cross-River State, Nigeria.

“The animals in all the experimental factions” collected 450mg/kg body weight of the Zidovudine. The drug was dissolved in 150mls of distilled water and administered once daily to group C, D, and E for a period of seven days, while group B received the drug for a period of three weeks.

### 2.4 Determination of Body Weights of Experimental Animals

The final weights of the animals were recorded a day after the last dose of administration.

### 2.5 Collection of Experimental Specimen

At the end of the administration, the animals were anaesthesised using chlorofoam inhalation technique. The skull was opened and the hypothalamus and pituitary gland were dissected out and immediately fixed in 10% buffered formalin for histological, histochemical methods. The abdomen was dissected out to get the adrenal gland, and thereafter the organs were fixed in 10% formalin for histological staining

methods. Blood collections were gathered through heart puncture for chemical biologically analysis on the last day (Day 21) of administration of the herbal extracts.

#### 2.5.1 Histochemical technique (cresoyl fast violet staining method)

Sections were brought to distilled water, and stained in 1% aqueous solution of toluidine blue and was heated to 50-60%, and kept at the temperature for 30 to 60 minutes. The sections were rinsed rapidly in 95% alcohol, and differentiated with absolute alcohol until the nissl granules are sharply defined which usually takes a few seconds. And finally sections were cleared in xylene and mounted in DPX.

#### 2.5.2 Tinctorial methods for demonstration of anterior pituitary cells [32]

Periodic Acid Schiff orange G (PAS-Orange G) method for the demonstration of anterior pituitary cells. Sections were dewaxed and brought to distilled water; these sections were treated with periodic acid for 5 minutes, “cleansed adequately” with numerous alteration of cleaned water closed with substances Schiff for 15minutes. The slides were then cleaned in moving water for 5-10 minutes, nuclei was stained with Harris haematoxylin, differentiated in acid alcohol, blued and washed in water. Sections were stained in 2% orange G in 5% phosphotungistic acid for 20 seconds. Differentiation was done using tap water until sections was microscopically yellow; sections were finally dehydrated, cleared and mounted in DPX.

#### 2.5.3 Statistical Analysis for Malondialdehyde and Glutathione per-oxidase

Data were expressed as mean ± S.E.M. Stastical analysis was carried out using one-way analysis of variance (ANOVA) with significance expressed as $p<0.05$.

## 3. RESULTS

### 3.1 Body Weights

“There were no significant (decrease or increase) ($p<0.05$)” in the body weights of the treated rats compared with the negative control group (Table 1). Positive controlgroup showed a significant decrease in body weights of rats at ($P<0.05$), while the groups that received the herbal extract witnessed an increase in body weight.

### 3.2 Glutathione per-oxidase Activity (GPX)

The result showed an increase in GPX activity for groups C, D, and E when compared with groups A and B that were significantly reduced. However group C showed a significant increase in GPX activity when compared with the other experimental groups at $p<0.05$.

### 3.3 Malondialdehyde Activity

Results showed an increase in the levels of MDA for group A when compared to other experimental groups such as group B, C, D, and E. With group showing a significant decrease in the levels of MDA.

**Table 1. Showing body weights of different experimental groups**

| Day 0 | Negative control | Positive control | Spondias group | Azadirachta group | Combined group |
|---|---|---|---|---|---|
| Day 0 | 147±21.7 | 175±17.34 | 190±11.74 | 183±12.42 | 212±40.00 |
| Day 7 | 175±16.6 | 148±17.30 | 168±8.37 | 156±16.16 | 178±32.11 |
| Day 14 | 187±21.7 | 142±16.48 | 181±6.52 | 162±16.43 | 214±37.82 |
| Day 21 | 209±20.12 | 134.6±16.2 | 179.4±9.00 | 169±17.80 | 220±38.1 |

*Values are expressed as mean ± sem, n=5, the values were significant at p< 0.05 for all groups.*

**Table 2. Showing comparison of glutathione peroxidase in different experimental animals**

| Animal group | Mean ± standard deviation |
|---|---|
| Positive control group | 149.33 ± 21.11 |
| Negative control group | 132 .13 ± 19.99 |
| Spondias group | 291.93 ± 64.90 |
| Azadirachta indica | 253 ± 24.35 |
| Combined group | 269.90 ± 23.13 |

**Table 3. Showing comparison of malondialdehyde (MDA) concentration in different experimental groups**

| Animal groups | Mean ± Standard deviation |
|---|---|
| Positive control | 4.81 ± 0.12 |
| Negative control | 7.28 ± 0.52 |
| Spondias group | 5.36 ± 0.24 |
| Azadirachta group | 6.44 ± 0.13 |
| Combined group | 4.43 ± 0.29 |

### 3.3 Histological Observation

**Hypothalamus:** Histological observations of the Hypothalamus of male wistar rats of group A,B,C,D, and group E was carried out using light microscopic evaluation. Group A of the rat hypothalamus the results showed prominent hypothalamic neurons, Sections of group B of rat hypothalamus revealed an increase in size of the neurons and presence of vacuoles, In group C of rat hypothalamus the sections revealed a reduction in size of the neurons and less vacuoles. Sections of group D of rat hypothalamus showed a reduction in the size of neurons with lesser vacuoles. While sections of group E of rat hypothalamus showed normal distribution of hypothalamic neurons with fewer vacuoles.

**Anterior Pituitary Gland:** Histological observations of the Rat pituitary gland of male wistar rats was carried out using light microscopic evaluation. Results of Group A showed prominent acidophilic and basophilic cells with presence of blood vessels, Sections ofGroup B showed less prominent acidophilic and basophilic cells and a presence of vacuoles , Sections of Group C showed few vacuoles, and less prominent of acidophilic and basophilic cells. Group D witnessed more prominent acidophilic and basophilic cells, and a presence of blood vessels.

While group E showed more prominent acidophilic and basophilic cells, with presence of blood vessels.

**Adrenal gland**

The cytoarchitecture of rat adrenal gland of male wistar rats was carried out using light microscopic evaluation. Results Group A showed prominent layers of the adrenal cortex which are the *Zona glomerulosa, Zona fasciculate* and *Zona reticularis* with the adrenal medulla, While Group B showed presence of haemorrhage in all the 3 layers of the adrenal cortex and the adrenal medulla.

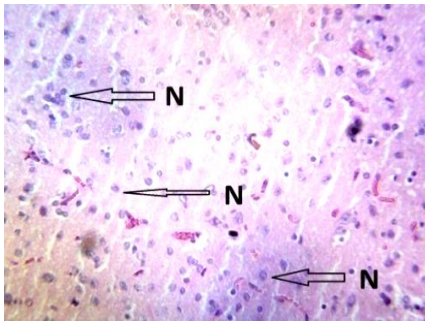
Group C witnessed prominent layers of the adrenal cortex, and lesser haemorrhage in the medulla, Group D witnessed prominent layers of the adrenal cortex, and less hemorrhage in the medulla, while group E showed prominent layers of the adrenal cortex, and less haemorrhage.

## 4. DISCUSSION

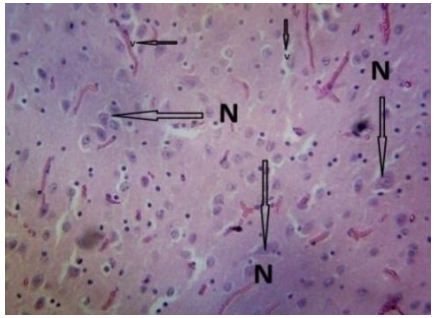
Oxidative stress can be defined as an imbalance between the production of reactive oxygen species and inability of biological system of anti-oxidants to detoxify the reactive intermediate or repair the resulting damage [11]. Zidovudine is an antiretroviral drug used to treat and prevent HIV/ AIDS, it is sold in combination with Lamivudine and this drug can be taken orally or by slow injection into a vein [33]. Its side effects include; anaemia, neutropenia, hepatotoxicity, cardiomyopathy, oxidative stress, apoptosis of muscle cells, and weight reduction in experimental animals [34,35,36].

The study shows group A to have an increase in body weight, while group B, C, D and E showed a reduction in body weight and loss of fur when induced with zidovudine drug. The observed weight reduction in the various experimental groups B,C D, and E, coincides with the previous studies on zidovudine by Lamperth et al. [36], which proved that experimental animals treated with zidovudine lost 10% of their original body weight by the end of the experiment that lasted for three months. Subsequent administration of methanolic leaf extracts of *Azadirachta indica* and *Spondias mombin* restored loss of fur and subsequently increased the weight of the experimental animals in groups C,D, and E. The restoration in weight of the animals with fur may be due to the anti-oxidative properties exhibited by both *Azadirachta indica* and *Spondias mombin*.

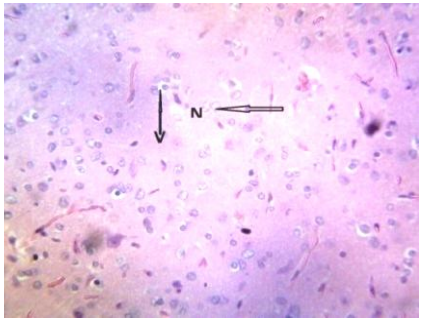
**Photomicrograph of Hypothalamus**



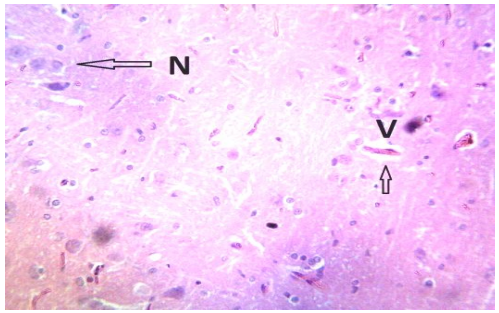

**Group A showing prominent hypothalamic neurons presence of enlarged neurons (N) of hypothalamus neurons.(N). H & E × 400.**



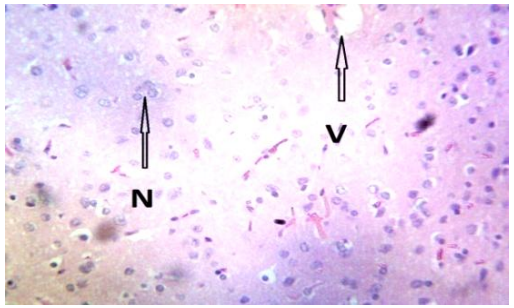

**Group B group B showed the presence of presence of enlarged neurons (N) with presence of vacuoles. H & E × 400**




**Group C showing a reduction in neuronal size(N) with lesser vacoules(V). H & E × 400**



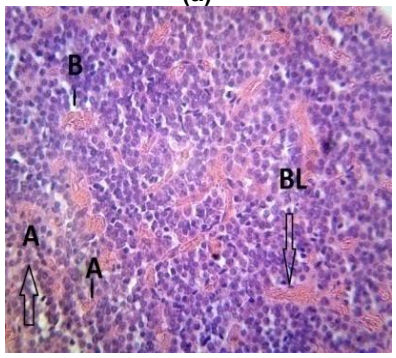

**Group D showing a reduction in neuronal size with less vacuoles, H & E × 400**




**Group E showing less vacuoles with a reduction in size of neurons. (H & E ×400)**

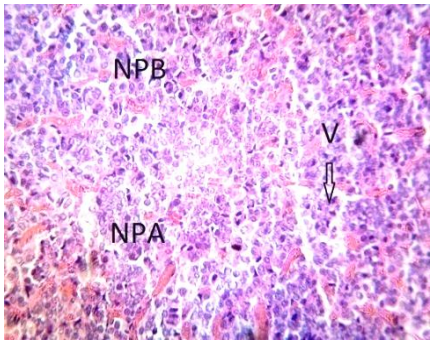
**Photomicrograph of pituitary gland**

**(a)**



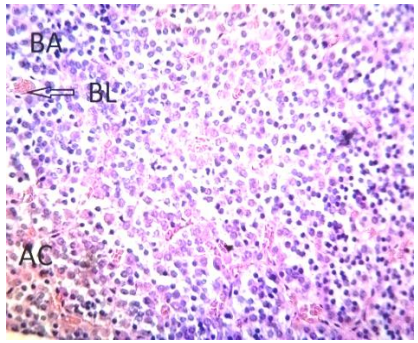

**Group A shows the presence of Acidophilic cells (AC), Basophilic cells (B) with presence Bloovessels (BL). (H&E×400).**

**(b)**



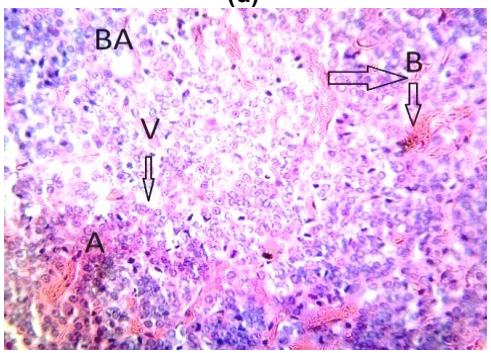

**Group B showed the presence of Non-prominence of acidophilic cells, basophilic cell with presence of vacuoles (V). (H&E×400).**

**(c)**



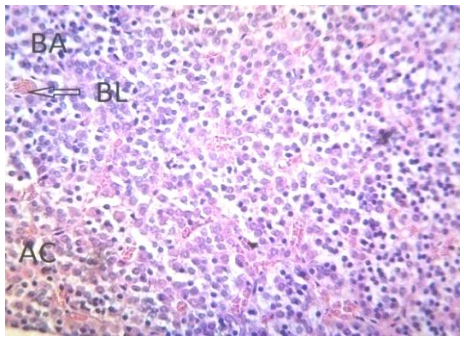

**Group C showed normal histology of acidophilic cells and basophilic cells with presence of blood vessels. (H&E×400).**

**(d)**




**Group D showed presence of vacuoles (V) with a slight distortion in the histology of acidophilic(A) and basophilic cells (B). (H&E×400).**

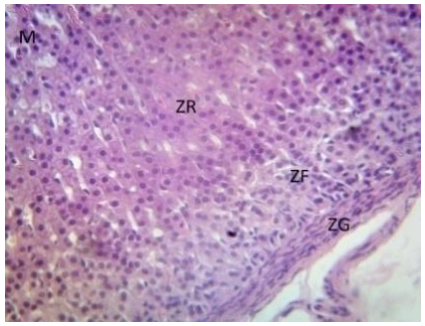
**(e)**




**Group E showed a normal histology of acidophilic cells, basophilic cells with the presence of blood vessels. (H&E×400).**

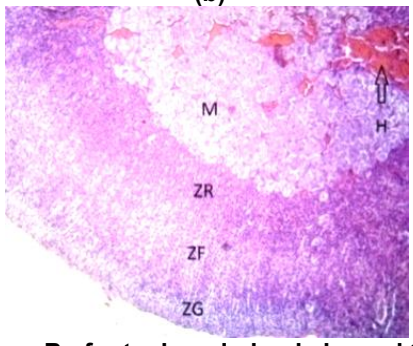
**Photomicrography of Adrenals**

**(a)**



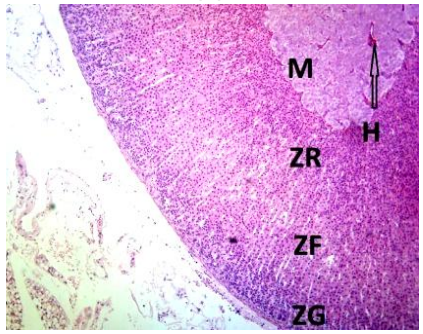

**Group A showed the 3 layers of the adrenal cortex which are Zona glomerulosa (ZG), Zona fasiculata (ZF), and Zona reticularis (ZR) with the medullar layer. H & E × 400**

**(b)**



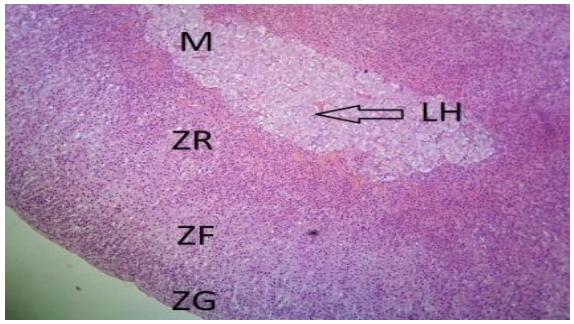

**Group B of rat adrenal gland showed the presence of vacuoles and hemorrhage in ZF, ZG and ZR of the adrenal cortex and the medullar layer. H & E × 400.**

**(c)**



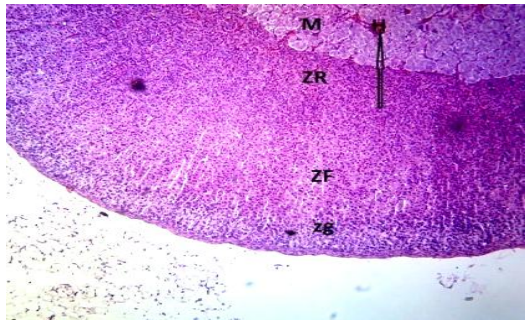

**Group C showed a reduction of vacuoles and hemorrhage in the 3 layers of the adrenal cortex and the medulla. H & E × 400.**

**(d)**




**Group D showed the presence of less hemorrhage in the 3 layers of the adrenal cortex and the medulla. H & E × 400**

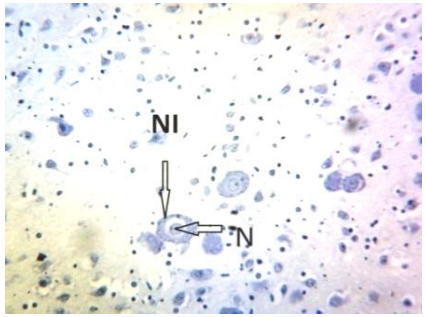
**(e)**




**Group E showing the presence of reduction of haemorrhage(H), in the Medulla (M), Zona reticularis(ZR), Zona fasiculata (ZF), and Zona glomerulosa (ZG). H & E × 400**

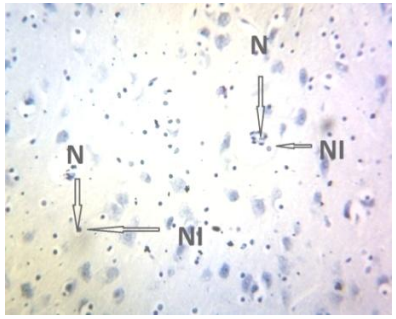
**Histochemical staining methods (cresoyl fast violet method)**

**(a)**



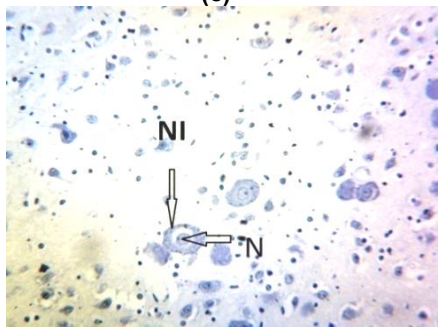

**Group A showed a prominent expression of the nissl substance (Nl) and normal arrangement of neuron (N). Mag × 400.**

**(b)**



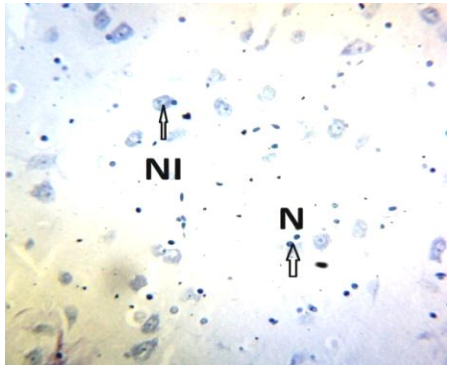

**Group B showed a reduction in the expression of nissl(Nl) substance. Mag × 400.**

**(c)**



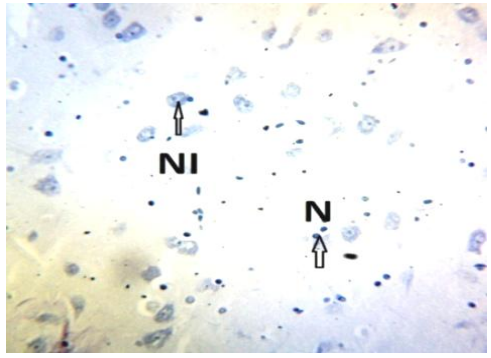

**Group Cshowed prominent expression of nissl substance (Nl). Mag × 400.**

**(d)**




**Group D showedmoreprominentexpressionofnissl substance. Mag × 400**

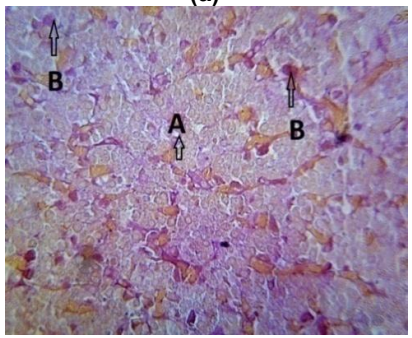
**(e)**




**Group Ealsoshowed prominent expression of nissl substance. . M.ag × 400**

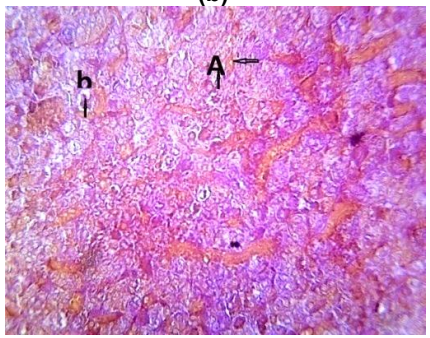
**Photomicrograph of Rat Anterior pituitary using Orange genyl stains**

**(a)**



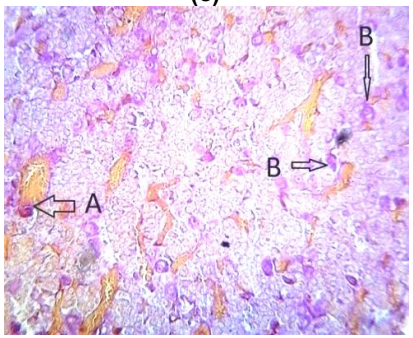

**group A of rat anterior pituitary glandshowed the presence of prominent acidophilic and basophilic cells. Mag × 400.**

**(b)**



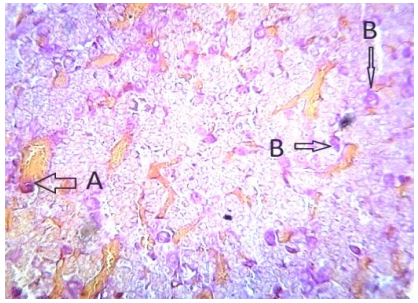

**Group B showed a reduction in the expression of orange G and also less prominence of acidophilic and basophilic cells. Mag × 400.**

**(c)**



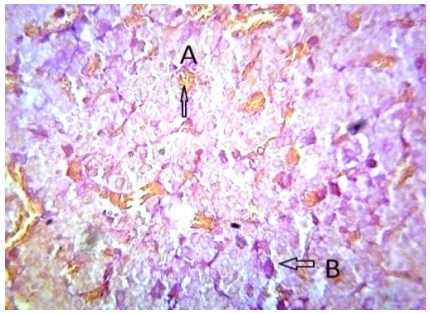

**Group C that received showed prominent acidophilic and basophilic cells, and a restoration in the expression of orange G. Mag × 400.**

**(d)**




**Group D that received showed prominent acidophilic and basophilic cells, and a restoration in the expression of orange G. Mag × 400.**

**(e)**




**Group E showed moderate expression of orange G. and prominent expression of acidophilic and basophilic cells . Mag × 400.**

Prominent hypothalamic neurons were witnessed in group A, group B showed less prominent hypothalamic neurons, degeneration of neurons, enlarged neurons with presence of vacuoles, group C showed lesser vacuoles, and prominence of neurons, group D showed prominent neurons, with lesser vacuoles and group E showed fewer vacuoles with prominent hypothalamic neurons. The findings from group B is in line with studies conducted by Loana et al. [37] who reported the presence of pyknotic nuclei, hypertrophy of neurons of the hypothalamus with the presence of vacuoles in their cytoplasm of laboratory animals when exposed to anakinetic stress. Therefore this may suggest a decrease in hypothalamic secretion of corticotrophin releasing hormone and also it may lead to impairment in the synthesis and release of neurotransmitters in the brain. Results of group C, D, and E shows that intake of anti-oxidants may have ameliorative effects on animals induced with oxidative stress and this is in line with studies conducted by Naoko et al. [38] who reported the effect of oxidative stress on the HPA axis of wistar rats and its prevention by Vitamin E, the study revealed that induction of oxidative stress can induce damage on the HPA axis and this may lead to dampening of the negative feedback mechanism of the HPA axis which resulted to an increase in the level of ACTH and corticosterone and hence homeostasis may be affected, but on treated with Vitamin E supplements, the results showed a decrease in the levels of ACTH and corticosterone. Intake of herbal extracts by group C, D, and E led to a reduction in the presence of vacuoles and neuronal degeneration. This however, may be due to the neuroprotective potentials of both herbal extracts when exposed to oxidative stress.

Further exposure of animals to anakinetic stress resulted to the increase in number of basophil cells and acidophilic cells, the glandular parenchyma showed the presence of vacuoles with dilated pericellular space which is an indication of adenohypophysis hormonal secretion [*37*]. The effects of anakinetic stress on the pituitary gland of experimental animals was studied and the result revealed an increase in number of basophil cells and acidophilic cells, the presence of vacuoles with dilated pericellular space was observed. This may be an indication of impairment in the secretion of adenohypophysis hormonal secretion [37].

Group A of the anterior pituitary gland of rats showed acidophilic and basophilic cells to be prominent and there were presence of blood vessels. Group B of the anterior pituitary gland of rats showed less prominent acidophilic and basophilic cells with presence of vacuoles. Presence of vacuoles in group B may be as a result of intake of zidovudine drug, which may be a sign of cell death. Group C results showed fewer vacuoles, prominent acidophilic and basophilic cells. Results of Group D showed prominence of acidophilic and basophilic cells with presence in blood vessels. While results of Group E showed prominent acidophilic and basophilic cells with presence of blood vessels. The presence of of reduced vacuoles in groups C,D, and E may be as a result of the effect of intake of herbal extracts by experimental animals.

Observations from Orange G stains for group A of the rat anterior pituitary showed prominent acidophilic and basophilic cells with prominent expression of orange G, Group B result showed a reduction in the expression of orange G and less prominence of acidophilic and basophilic cells, Group C results showed a restoration of the orange G, Results of group D revealed prominent acidophilic and basophilic cells, and a restoration of the orange G, and Group E showed a restoration of the orange G, with prominent acidophilic and basophilic cells.

The study carried on the pituitary gland corresponds to the finding of Feng et al. [39], who reported the effect of heat stress on the cytoarchitecture of the anterior lobe of mouse pituitary gland, results revealed the presence of rapid proliferation of anterior pituitary cells, and apoptosis due to exposure of heat stress. Greave [41]. Further investigated the effect of exposure of pituitary gland to certain toxicants; showed the cells of the anterior pituitary gland to undergo hypertrophy, hyperplasia and atrophy; these changes in the cells of the anterior pituitary gland is due to prolonged hormonal imbalance, and also an altered feedback pathway to the pituitary gland.

Results of adrenal gland group A of rat showed presence of clustered secretory cells, prominence of the 3 layers of the adrenal cortex which are the Zona glomerulosa, Zona Fasiculata, and Zona reticularis with the adrenal medulla. The presence of haemorrhage in group B, C, D and E work corresponds to the findings of [42] which reported that under stressful

conditions, ACTH secretion increases and this leads to stimulation of arterial blood flow of the adrenal gland that may exceed the limited venous drainage capacity of the organ and cause hemorrhage in all the 3 layers of the cortex with the medulla. Group B showed the presence of hemorrhage in the zona reticularis and in the medulla. While Group C, D and E showed a reduction of haemorrhage in the reticularis and medulla with a prominence of the layers of the adrenal cortex. The restorative potentials of *Azadirachta indica* and *Spondias mombin* corresponds with the findings of Akabue et al., Nworu et al. [43,44] which showed the stem of *Azadirachta indica* to be useful in the treatment of wounds and scars.

Further studies on *Spondias mombin* also showed its usefulness in the treatment of inflammatory conditions, wounds, and infections, Therefore the presence of haemorrhage may be due to the intake of zidovudine drug by experimental animals while a reduction in haemorrhage may be due to the restorative potentials of both herbal extracts.

The neurons are functional units of the nervous tissue and can be identified using special stains like cresyl-fast violet which is used to stain nissl substance [45]. Certain drugs, chemicals, toxins, lack of oxygen can lead to alteration of in the staining intensities of Nissl substance and this has shown to interfere with normal cellular metabolism [46]. Decrease in staining of the nissl substance may be due to chromatolysis which is the migration of nissl substances towards the periphery of the soma due to either trauma or due to other exogenous agents [45]. Decrease in staining intensities of nissl substance may results to loss of function of the protein synthesising ability of the neurons, and since protein is the working molecules of the cells, this might ultimately result in the death of cells.

Cresoyl Fast Violet studies for group A that showed the prominence in the staining intensities of the nissl substance may be an indication of high level of protein synthesis, while a reduction in the staining intensities of nissl substance in Group B, may be due to harmful effects of zidovudine drug and this is in lineStudies conducted by Adjene et al. [47], who reported that chronic administration of effavirenz to experimental animals led to a depletion of nissl substances in the superior colliculus of treated rats which may be as a result of its harmful effect on neuronal intergrity and thus, a decrease in nissl substance might result to a decrease in cellular metabolism.

Malondialdehyde (MDA) is one of the final products of lipid peroxidation in cells, and an increase in the production of free radicals causes over production of malondialdehyde and therefore a high level of MDA is seen as a marker of lipid peroxidation and oxidative stress [48].

Studies have shown that elevated levels of malondialdehyde in the brain of wistar rats is an indication of deterioration in the cellular intergrity of the cerebral membrane. Subsequent administration of garlic extract by experimental animals exposed to oxidative stress led to a reduction in the levels of malondialdehyde in the brain, this is because garlic have the potential of scavenging free radicals such as hydrogen peroxide, oxygen, and hydroxyl ions [34,40].

Results for statistical analysis of malondialdehyde (MDA) for group A showed a reduction in MDA levels, the increase level of MDA in group B may be due to the production of free radicals by the zidovudine drug and this corresponds to the studies done by Studies by Sun et al. [34] which showed that elevated levels of malondialdehyde is an indication of oxidative stress, and this may be an indication of deterioration in the cellular intergrity of the cerebral membrane. Groups C and D showed a decrease in the levels of MDA and this may be due to the presence of certain phytochemical constituents present in both plants , while group E showed more reduction in the level of MDA which may be as a result of synergistic effect of both plants extracts. This result corresponds with works carried out by Patel & Chu [49]. On the administration of garlic extract on experimental animals exposed to oxidative stress; the study revealed a reduction in the levels of malondialdehyde in the brain and this is because garlic have the potential of scavenging free radicals such as hydrogen peroxide, oxygen, and hydroxyl ions [49].

GPX is a major pereoxide scavenging enzymes which functions in the protection of an organism from oxidative damage; its biochemical function is to reduce lipid hydroperoxides to alcohols as well as reducing free hydrogen peroxide to water [50]. Assessment for Glutathione peroxidase (GPX) for group A showed a slight increase in the levels of GPX, group B showed a significant

decrease in the levels of GPX, this may be due to the presence of oxdative stress in the tissues. While group C, D and E showed a significant increase in the levels of GPX with groups D having a larger increase in the levels of GPX. These results correspond with the study conducted by Miyamoto et al. [50] on the exposure of tissues to oxidative stress, results of the study showed a decrease in the levels of GPX, whereas an increase in the levels of GPX activity shows its protection against cell damage by reactive oxygen species.

The reduction in the levels of malondialdehyde and an increase in the levels glutathione peroxidase may be due to the anti-oxidative potentials of *Azadirachta indica* and *Spondias mombin* on oxidative stress. Hence the study shows that single intake of either *Azadirachta indica* or *Spondias mombin* proved to be more effective in the treatment of induced oxidative stress of wistar rats when compared with the synergestic effect of both herbs.

## 5. CONCLUSION

Therefore the study revealed the efficacy of single administration of either *Azadirachta indica* or *Spondias mombin* in ameliorating the effects of induced oxidative stress when compared to the combined administration of both herbal extracts.

## ETHICAL APPROVAL

This study was approved by the Department Ethics Committee of the\University of Calabar, Calabar.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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