

Evaluating the Effects of Methanolic Leaf Extract of Neem Plant and Hog Plum on the Liver Histology of Zidovudine Induced-Oxidative Stress Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors COU, ORA and OIE 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 liver is an important organ which functions in the detoxification of drugs, toxins and metabolic waste products. It also plays a role in the maintenance of homeostasis. Exposure of the liver to oxidative stress leads to impairment of homeostasis which may result in malfunctioning of the liver. This study was carried out to assess the efficacy in the administration of herbal extract of *Azadirachta indica* (neem) or *Spondias mombin* (hog plum) singly or in combination when ameliorating the effects of oxidative stress in wistar rats Liver. The study was carried out using 25 male adult wistar rats weighing 180-280 g. The rats were divided into five 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 is the positive control group that received the administration of 450mg/kg body

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weight of zidovudine drug, Group C is the group that received 450 mg/kg body weight of zidovudine drug and 500 mg/kg body weight of *Azadirachta indica* group D received 450 mg/kg body weight of zidovudine and 500 mg/kg body weight of *Spondias mombin*, while group E is the group that received 450 mg/kg body weight of zidovudine drug and a combination of 500 mg/kg body weight of *Azadirachta indica* and *Spondias mombin* herbal extracts. 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 liver was fixed in 10% neutral buffered formal saline. Light microscopic evaluation of the liver showed the normal histology of the liver which includes the presence of central vein of the liver, hepatocytes, and sinusoids in group A, sections of group B witnessed a distortion in the liver histology which is witnessed by shrinkage of central vein of the liver, degeneration of hepatocytes and a reduction in sinusoids, group C showed a normal histology of central vein of the liver, hepatocytes; and dilatation of liver sinusoids. Group D showed a normal histology of hepatocytes, sinusoids, with central vein of the liver, while groups E showed dilatation in the central vein of liver, dilatation of liver sinusoids and a normal histology of liver hepatocytes. Therefore the result shows that single administration of methanolic leaf extract of *Azadirachta indica* or *Spondias mombin* herbal extracts was more potent in ameliorating the effects of zidovudine induced oxidative stress on the Liver histology when compared with the synergistic effect of both herbal extracts.

Keywords: *Azadirachta indica*; *Spondias mombin*; Zidovudine; liver histology.

1. INTRODUCTION

Medicinal plants are considered as healthy sources for the prevention of various oxidative stress related diseases [1], this is because they are rich in certain phytochemical constituents having anti-oxidative activities such as phenolic compounds and carotenoids [2]. Various sections and traditions make use of native substances as lone herbs, join of plants and union of herbs. Combination of herbs could lead to complications as numerous associations can happen within the person constituent. Complications may arise because of numerous constituent in the native extracts [3]. However the impacts from plant-plant association are likely uncertain and complex [4-12]. The liver is an important organ in the body which functions in the detoxification of metabolic waste products, various drugs and toxins. It also functions in the destruction of worn out red blood cells and reclaims their constituents, it also functions in the metabolism and removal of drugs, It plays a role in the achievement of homeostasis by detoxification of drugs through the aid of metabolizing enzymes [13-16]. Liver is among the organs attacked by reactive oxygen species [17]. This may be because when the liver is exposed to the effects of inducing oxidative stress certain cells like parenchymal cells, kupffer cells, and hepatic stellate cells and certain organelles such as the mitochondria, microsomes, and peroxisomes becomes affected, and may lead to an increase in the production of apoptosis and inflammation [18-20].

It has been reported that vegetables, fruits, herbs and plant extracts are traditionally used for the treatment of liver diseases and so it is of importance to add vegetables to our diet as it plays a role in the detoxification of harmful substances present in the liver [21].

Azadirachta indica (neem tree) is a native plant of South eastern Asia, and it is distributed in India and other neighboring countries [22]. It is called dogonyaro in Hausa, and Ogwuakuma in Igbo [23]. *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 nimbinat, gedunin, salannin and quercetin [24]. *Azadirachta indica* is rich in phytochemical constituents like azadirachtin, nimbolide and ascorbate which possess significant anti-oxidant properties, that enables it to scavenge free radicals present in the body [25].

Spondias mombin belongs to the family *Anacardiaceae*, and it is one of the medicinal herbs in southern Nigeria [26]. It has several names; it is termed English in plum hog, Yoruba akika, tsardamaster in Hausa, Chabbuh in Fulani and nuskakara in Efik [27]. *Spondias* also possess anthelmintic, anti-oxidant, antimicrobial and anti-inflammatory actions, sedative and anxiolytic potentials [25-30]. Therefore this study was carried to evaluate the effects of oxidative stress on the histology of the Liver of Adult male wistar Rats so as 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 *Azadiratcha 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 obtained was filtered through whatmann paper 1 and the filtrate was evaporated to dryness on rotary evaporator at (50 °C). The extract was 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 Wistar 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 of average weight 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 chow, **Group B** is the Positive

control group that was induced with 450 mg/kg body weight of zidovudine drug for a period of three weeks. **Group C** is the Experimental group that was induced with 450 mg/kg body weight of zidovudine drug for a period of one week and received 500 mg/kg body weight of *Azadiratcha indica* for a period of two weeks. **Group D** represents Experimental group that was induced with 450 mg/kg body weight of zidovudine drug for a period of one week and received 500 mg/kg body weight of *Spondias mombin* for a period of two weeks. While **Group E** Experimental group received 450 mg/kg body weight of zidovudine drug for one week and 500 mg/kg body weight of *Azadiratcha indica* and *Spondias mombin* for a period of two weeks. At the end of the administration, the animals were anaesthetized 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.

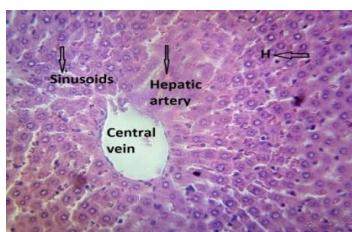
The animals in all the experimental faction collected 450 mg/kg body weight of the Zidovudine. The drug was dissolved in 150 mls of distilled water and administered once daily to group C, D, and E for a period of seven days, while group B received the drug for a period of three weeks.

2.4 Collection of Experimental Specimen

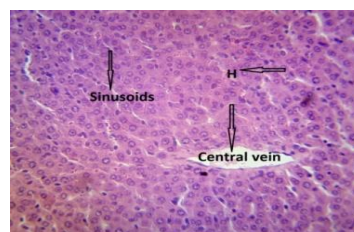
At the end of the administration, the animals were sacrificed using chlorofoam inhalation technique. The abdomen was dissected out to access the Liver for normal histological procedure.

3. RESULTS

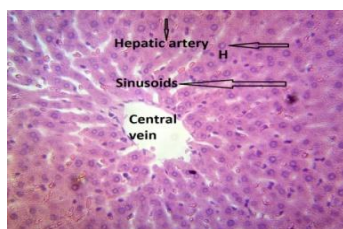
Histological Observation of the Liver:



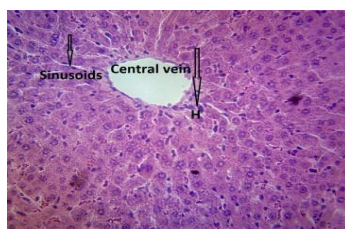
Photomicrograph of group A of rat Liver showing the presence of central vein, hepatic artery, sinusoids and hepatocytes (H). (H & E) × 400



Photomicrograph of group B Liver revealing the presence of shrinkage of central vein of the liver, degeneration of hepatocytes, with reduction sinusoids when compared to group A. (H & E) × 400



Photomicrograph of group C Liver showing a normal histology of central vein of the liver, hepatocytes; and dilatation of liver sinusoids (H & E) ×400



Photomicrograph of group D Liver showing a normal histology of the central vein of the liver, hepatocytes(H) and sinusoids. (H & E) × 400



Photomicrograph of group E Liver showing the presence of enlarged central vein of the liver, dilated sinusoidal space, and the presence of normal hepatocytes (H). (H & E) × 400

4. DISCUSSION

This study was carried out to assess the effect of Zidovudine drug on the histology of the rat liver in order to compare the efficacy of single administration of methanolic extracts of *Azadirachta indica* (neem) or *Spondias mombin* (hog plum) to a combination of both herbal extracts. In this study group A of the rat liver showed the presence of central vein of the liver, presence of hepatocytes, sinusoids and hepatic artery. Group B showed the presence of shrinkage in the central vein of the liver, degeneration of hepatocytes with a degeneration of sinusoids. Groups C witnessed a normal central vein, dilatation of sinusoids while group D witnessed a normal central vein with clusters of hepatocytes, restoration in the degenerated hepatocytes and sinusoids with the presence of hepatic artery. While Group E showed an enlargement of central vein of the liver presence of restored hepatocytes with and enlarged sinusoids.

Group B showed shrinkage of the central vein of the liver which is in line with studies carried out by Eweka et al. [31] showed a dilatation of the central vein and a distortion of the cytoarchitecture of the liver when treated with monosodium glutamate. Singh [32] reported that distortion in the cytoarchitecture of the liver could be associated with functional changes that may be detrimental to health. Willie [33] also reported that dilation and distortion of hepatocytes and sinusoids of the liver as a result of intake of monosodium-glutamatte may affect the haematopoietic functions of the liver. Sections of Group C showed a restoration of hepatocytes, dilatation of sinusoids and a normal central vein while group D of Rat liver showed a restoration of hepatocytes, central vein and dilatation of sinusoids were observed. The result of Group C

of rat liver is in line with the study conducted by Shirish [34] on the hepatoprotective effect of *Azadirachta indica* when induced by carbon tetrachloride, revealed the presence of necrosis in the liver, presence of vacuoles in the hepatocytes and dilation of sinusoids. Further treatment with *Azadirachta indica*(neem) leaf extract led to a recovery of hepatocytes and any alteration caused by carbon tetrachloride. Also Ubah [35] carried out a study on the HPA axis of Zidovudine stress induced wistar Rats proved that single administration of methanolic leaf extract of *Azadirachta indica* or *Spondias mombin* proved a restorative effect on the Hypothalamus, Pituitary, and Adrenal glands when exposed to oxidative stress. Results of Group D of rat liver is similar studies carried out by [36] on the hepatoprotective and anti-oxidant effects of *Spondias mombin* leaf and stem extracts upon carbon tetrachloride induced hepatotoxicity and oxidative stress. The study reported that animals treated with carbon tetrachloride witnessed an increase in the levels of MDA, AST, ALT and ASP. This therefore shows that extract of *Spondias mombin* may serve as a promising herb for the treatment of hepatic damage.

Results of Group E showed the presence of enlargement of central vein of the liver, reduced sinusoidal space and a restoration of hepatocytes. Enlargement of central vein of the liver may be a sign of serious implication in the function of the liver [37]. Also Ekong et al. [38] reported the presence of distorted sinusoids which may also impair the functions of the liver.

5. CONCLUSION

Therefore, this study proves that single administration of either *Azadirachta indica* (neem) or *Spondias mombin* (hog plum) extracts

had a more restorative effect on the liver histology when exposed to zidovudine induced oxidative stress than the combined herbal extracts.

CONSENT

It is not applicable.

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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