***Original Research Article***

# Evaluating The Effect of methanolic leaf extract of *Azadirachta indica and Spondias mombin* on the Liver Histology of Zidovudine induced-oxidative stress wistar Rats.


**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 access the efficacy in the administration of herbal extract of *Azadiratcha indica* or *Spondias mombin 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 180-280g. 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 weight of zidovudine drug, Group C is the group that received 450mg/kg body weight of zidovudine drug and 500mg/kg body weight of *Azadiratcha indica* group D received 450mg/kg body weight of zidovudine and 500mg/kg body weight of *Spondias mombin,* while group E is the group that received 450mg/kg body weight of zidovudine drug and a combination of 500mg/kg body weight of *Azadiratcha 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 were fixed in 10% neutral buffered formal saline. Light microscopic evaluation of the liver showed the presence of central vein of the liver, hepatocytes, and sinusoids in group A, sections of group B showed shrinkage of central vein of the liver, degeneration of hepatocytes and sinusoids, group C showed presence of dilated sinudoids, restoration of degenerated hepatocytes, and central vein of the liver, results of 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 with degeneration of liver hepatocytes. Therefore the result shows that single administration of methanolic leaf extract of *Azadiratcha indica* was more potent in ameliorating the effects of oxidative stress when compared with the synergistic effect of both herbal extracts.



KeyWords: of *Azadiratcha indica, Spondias mombin,* Zidovudine , Liver Histology


## 1.0 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 carotenids [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],[5], [6],[7], [8], [9],[10], [11], [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]; [14], [15], [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 mitochoindria, microsomes, and peroxisomes becomes affected, and may lead to an increase in the production of apoptosis and inflammation. [18], [19], [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].

*Azadiratcha 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]. *Azadiratcha 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 [24]. *Azadiratcha 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 [25].

*Spondias mombin* belongs to the family *Anarcadiaceae*, 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 anthelminthic, anti-oxidant, anti-microbial and anti-inflammatory actions, sedative and anxiolytic potentials [25, 26 ,27,28,29,30]. Therefore this study was carried to evaluate the effects of oxidative stress on the histology of the kidney 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.

**2.1: 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□). The extract were preserved in clean glass container for further use.

**1.2 :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.

**2.3: 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 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 C** is 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 *Azadiratcha 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 *Azadiratcha indica* and *Spondias mombin* for a period of two weeks. At the end of the administration, the animals were anaesthesized using chlorofoam inhalation technique.

**2.4: 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 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.

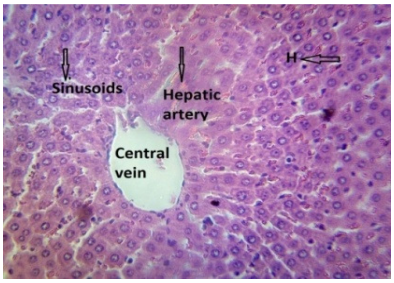
**2.5 Determination of body weights of experimental animals**

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

**2.6: Collection of experimental specimen**

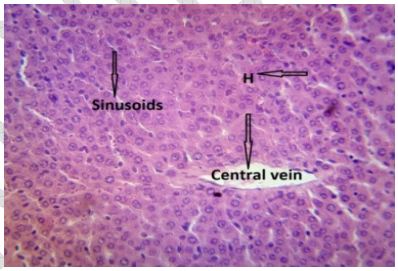
At the end of the administration, the animals were anaesthesized using chlorofoam inhalation technique.. The abdomen was dissected out to access the Liver for normal histological procedure..

**3.0 Histological Observation of the Liver**

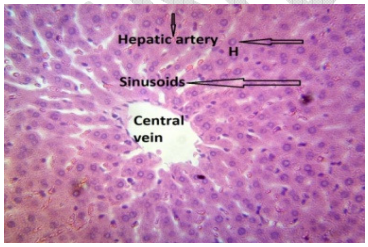



Photomicrograph of group A of rat Liver showing the presence of central vein, hepatic artery, sinusoids and hepatocytes (H).

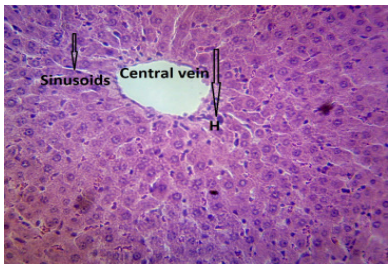
(H & E) × 400 .



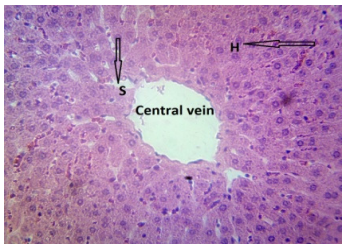

Photomicrograph of group B Liver revealing the presence of shrinkage of central vein of the liver, degeneration of hepatocytes, with reduced sinusoidal space when compared to group A. (H & E) × 400.




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




Photomicrograph of group D Liver showing a restoration in the central vein of the liver, restoration of hepatocytes(H) with a restoration of sinusoids. (H & E) × 400.




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

## 4.0 Discussion

This study was carried out to assess the effect of Zidovudine drug on the histology of the rat liver in other to compare the efficacy of single administration of methanolic extracts of Azadiratcha indica or Spondias mombin 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 and dilatation of the central vein of the liver which is in line with studies carried out by [31] showed a dilatation of the central vein and a distortion of the cytoarchitecture of the liver when treated with monosodium glutamate. [32] reported that distortion in the cytoarchitecture of the liver could be associated with functional changes that may be detrimental to health. [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 [34] on the hepatoprotective effect of *Azadiratcha 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

*Azadiratcha indica* leaf extract led to a recovery of hepatocytes and any alteration caused by carbon tetrachloride. Also [38] carried out a study on the HPA axis of Zidovudine stress induced wistar Rats proved that single administration of methanolic leaf extract of *Azadiratcha 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 [35] 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 [36]. Also [37] reported the presence of distorted sinusoids which may also impair the functions of the liver. Therefore, this study proves that single administration of either *Azadiratcha indica* or *Spondias mombin* extracts proved ameliorative effect when compared to the combination of herbal extracts with spondias mombin having a more restorative effect in the histology of zidovudine-stressed induced wistar Rats.

## Ethical Approval

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

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