

Effects of methanolic leaf extracts of *Azadirachta indica* and *Spondias mombin* on the Histology of the kidneys of Zidovudine stress induced wistar Rats.

ABSTRACT

The kidneys play a role in the maintenance of homeostasis by ensuring the excretion of waste and toxic substances from the body. Oxidative stress could be defined as an imbalance between the production of reactive oxygen species and an inability of the body system to scavenge the presence of free radicals. Intake of certain drugs and toxic substances exposes the Kidneys to oxidative stress effects and this may lead to impairment of homeostasis and malfunctioning of the Kidney. This study was carried out to assess the efficacy in administration of single herbal extracts of either *Azadirachta indica* or *Spondias mombin* when compared to the combination of both herbal extracts in ameliorating the effects of oxidative stress in wistar Rats Kidney. The study was carried out using 25 male adult wistar rats of weight 180-200g, the animals were randomly selected and were designated into groups A (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 *Azadirachta indica* group that received 450mg weight of zidovudine drug and 500mg/kg body weight of methanoic leaf extract /kg body, group D is the *Spondias mombin* group that received 450mg/kg body weight of zidovudine drug and 500mg/kg body weight of methanolic leaf extract and group E received a 450mg/kg body weight of Zidovudine and a combination of 500mg/kg body weight of both methanolic leaf extracts of *Azadirachta indica* and *Spondias Mombin* leaf . The administration was carried out once a day using orogastric tube for a period 21 days. At the end of the administration, the rats were sacrificed using chloroform inhalation technique and the kidney was fixed in 10% neutral buffered formal saline. Light microscopic evaluation of the Kidney showed normal histological appearance of the kidney in group A as witnessed by the presence of glomerulus, proximal convoluted tubule (PCT), distal convoluted tubule (DCT), bowmans space (BS), while group B witnessed alterations in the histology of the liver as shown by the presence of haemorrhage in the glomerulus, shrinkage in the proximal and distal convoluted tubule and shrinkage of the bowmans space, group C and D witnessed a restoration of the Kidneys histology as evidenced by a reduction of haemorrhage in the glomerulus and shrinkage PCT and DCT. Group E showed an enlargement of the Bowmans space and shrinkage of the PCT and DCT. Hence the results proved the efficacy of single administration of herbal extracts in ameliorating the effects of oxidative stress when compared with the combination of the herbal extracts.

Key Words: *Azadirachta indica*, *Spondias mombin* leaf, kidneys, Zidovudine

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 carotenoids [2]. Medicinal plants derived anti-oxidants can protect renal damage through reduction of lipid peroxidation and an

40 increase in the levels of anti-oxidants. [3]. Various sections and traditions make use of native
41 substances as lone herbs, join of plants and union of herbs. Combination of herbs could lead to
42 complications as numerous associations can happen within the person constituent.
43 Complications may arise because of numerous constituent in the native extracts. [4]. However
44 the impacts from plant-plant association are likely uncertain and complex [5], [6], [7], [8], [9],
45 [10], [11], [13], [14]. Oxidative stress can be defined as a disproportion among the system
46 display of active kind air and a functional body capacity to remove the active intermediate or to
47 restore the outcome injury [15]. It is caused when the existence of liberal substance overwhelms
48 the free scavenging mechanism of anti-oxidants [16]. Oxidative stress is also an important factor
49 which can contribute to kidney damage by increasing the production of oxidants, especially
50 insufficiency of anti-oxidants defense system [17]. Oxidative stress induced damage on the
51 kidney is associated with an increase in the production of reactive oxygen species [18].
52 The kidneys are paired bean shaped organs located on the posterior abdominal cavity [19]. It
53 functions in the maintenance of homeostasis through the excretion of metabolic waste products,
54 regulation of extracellular volume, as well as regulation of electrolyte composition and acid
55 base balance [20]. Exposure of the Kidney to several drugs, toxic xenobiotics, or chemicals can
56 cause toxic damage to the kidney due to its high rate of blood flow (21). *Azadirachta indica*
57 (neem tree) is a native plant of South eastern Asia, and it is distributed in India and other
58 neighboring countries [22]. It is called dogonyaro in Hausa, and Ogwuakuma in Igbo [23].
59 *Azadirachta indica* plays therapeutic role in the management of health due to the presence of
60 rich source of various types of ingredients. Most important active chemical components of
61 *Azadirachta indica* is Azadirachtin, nimbolin, nimbin, nimbol, sodium nimbinat, gedunin,
62 salannin and quercetin [24]. *Azadirachta indica* is rich in phytochemical constituents like

63 **azadirachtin**, nimbolide and ascorbate which possess significant anti-oxidant properties, that
64 enables it to scavenge free radicals present in the body [25].

65 *Spondias mombin* belongs to the family **Anarcadiaceae**, and it is one of the medicinal
66 herbs in southern Nigeria [26]. It has several names; it is termed English in plum hog, Yoruba
67 akika, tsardamaster in Hausa, Chabbuh in Fulani and nuskakara in Efik [27]. *Spondias* also
68 possess anthelmintic, anti-oxidant, anti-microbial and anti-inflammatory actions, sedative and
69 anxiolytic potentials [25, 26, 27, 28, 29, 30]. Therefore this study was carried to evaluate the
70 effects of oxidative stress on the histology of the kidney of Adult male wistar Rats so as to
71 compare the impacts of single administration of herbal extracts with the combination of herbal
72 extracts in ameliorating the effects of oxidative stress.

74 **2.1: Materials and methods**

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

83 **1.2: Animals**

84 This study was approved by the Department Ethics Committee of the University of
85 Calabar, Calabar. Twenty-five male adult Wister Rats with an average weight of 200 g were

86 bred in the animal house of the department of Anatomical Sciences and were used for this study.
87 The rats were fed with rat chow, water ad libitum.

88 **2.3: Experimental Protocol.**

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

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

101 **2.4: Stress Induction.**

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

105 The animals in all the experimental faction collected 450mg/kg body weight of the Zidovudine.

106 The drug was dissolved in 150mls of distilled water and administered once daily to group C, D,
107 and E for a period of seven days, while group B received the drug for a period of three weeks.

108 **2.5 Determination of body weights of experimental animals**

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

110 2.6: Collection of experimental specimen

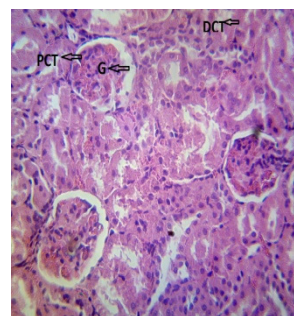
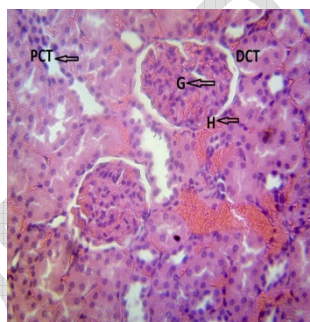
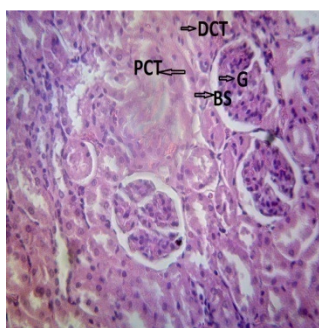
111 At the end of the administration, the animals were anaesthetized using chlorofoam inhalation
112 technique. The abdomen was dissected out to access the Kidney which was located on top of
113 each Adrenal gland.

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117 3.0 Histological Observation of the Kidney



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119 Photomicrograph of normal
120 histology of negative control kidney
121 group showing the presence of the
122 glomerulus (G), distal convoluted
123 tubule (DCT), proximal convoluted
124 tubule (PCT), and bowmans space
125 (BS). H and E x400.

119 Photomicrograph of positive control
120 group of Rat kidney showing the
121 presence of haemorrhage, shrinkage
122 of distal and proximal convoluted
123 tubule and shrinkage of bowman
124 space (BS). H and E x400.

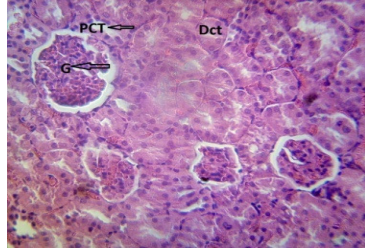
119 Photomicrograph of *Azadirachta*
120 *indica* group kidney group showing
121 Presence of glomerulus, and a
122 restoration of haemorrhage in the
123 distal convoluted tubule (DCT),
124 proximal convoluted tubule (PCT),
125 with a normal bowmans space. H and
126 E x400.

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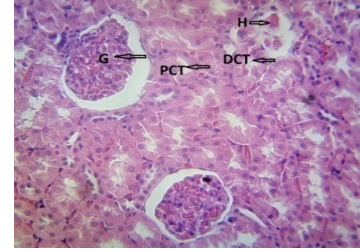
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Photomicrograph of *Spondias mombin* group showing Presence of glomerulus, and a restoration of haemorrhage in the distal convoluted tubule (DCT), proximal convoluted tubule (PCT), with a normal bowmans space. H and E ×400.



Photomicrograph of combined kidney group showing the presence of dilated bowmans space, with presence of shrinkage in the glomerulus, pct and dct of Rat kidney. (H&E ×400).

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138 **4.0 Discussion**

139 This study was carried out to assess the effect of Zidovudine drug on the histology of the rat
140 kidney in other to compare the efficacy of single administration of methanolic extracts of
141 *Azadirachta indica* or *Spondias mombin* to a combination of both herbal extracts.

142 Exposure of the kidney to certain chemical agents or drugs could be manifested by the presence
143 of vascular congestion (glomerulus), inflammatory cell infiltration with the presence of hyaline
144 globule in the collecting tubule [31].

145 Light microscopic evaluation of the Kidney showed normal histological appearance of the
146 kidney in group A as witnessed by the presence of glomerulus, proximal convoluted tubule
147 (PCT), distal convoluted tubule (DCT), and bowmans space (BS), while group B witnessed
148 alterations in the histology of the liver as shown by the presence of haemorrhage in the
149 glomerulus, shrinkage in the proximal and distal convoluted tubule and shrinkage of the
150 bowmans space, group C and D witnessed a restoration of the Kidneys histology as evidenced

151 by a reduction of haemorrhage in the glomerulus and shrinkage PCT and DCT. Group E showed
152 an enlargement of the Bowmans space and shrinkage of the PCT and DCT.

153 Results of group B and E is similar to the studies carried out by [32] which reported the
154 presence of wider capsular space, congested glomerular tufts, and degeneration of the tubules
155 when treated with cisplatin. Also results of group B,C,D and E is similar to works carried out
156 by [Aboonabi et al. \[33\]](#) on the ameliorative effect of pomegranate on the histopathology of the
157 Kidney of diabetic induced oxidative stress. The study revealed the presence of shrinkage and
158 lesions in the bowmans capsule when exposed to oxidative stress, but intake of pomegranate
159 herbal extract rich in anti-oxidants led to a reversal in histological changes of the kidney.

160 The restoration in the histology of group C, and D may be due to the presence of anti-oxidants
161 present in the above herbal extracts, while the widening of the bowman space may be due to the
162 effect of the drug on the kidney histology. Studies carried out by [Dhar et al \(1996\)](#) *?not*
163 *according to the format of the journal?* showed that methanolic leaf extract of *Azadirachta indica*
164 can ameliorate the effects of oxidative stress on the kidney. This may be due to its
165 antinephrotoxic potential.

166

167 **Conclusion**

168 The results of the study proved that single administration *Azadirachta indica* and *Spondias*
169 *mombin* ameliorated the effects of oxidative stress on the Kidney histology of male wistar rats
170 when compared with the combination of both herbal extracts in ameliorating the effects of
171 oxidative stress on the kidney. The effects of combined herbal therapy could not be compared
172 with the single administration of the herbs, this may be due to interaction between the
173 phytochemical components of both herbal extracts.

174 Ethical Approval

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

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