

Research Article,

## Effect of Aqueous Extract of *Alstonia Boonei* on Liver Enzymes and Its Anti-Inflammatory Activity on Formalin- Induced Arthritic Wistar Rats

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### Abstract:

The anti-inflammatory effect of the aqueous extract of *Alstonia boonei* on the level of liver biomarker in the plasma of normal and arthritic rats was evaluated using the formalin-induced arthritis inflammation tests. Thirty (30) adult female Wistar rats were divided into six groups of five rats each. Group 1 as control, group 2(formalin induced group) while groups 3, 4 and 5 were respectively gavage with 150mg/kg, 300mg/kg and 600mg/kg body weight of the extract daily while group 6 was given reference drug( 5mg/kg body weight of indomethacin for 5 days). After formalin treatment, the paw thickness in formalin-induced group was increased compared with control group. The levels of TNF-alpha, CRP, liver enzymes were increased with severe chronic inflammation sign at histopathologically observation. The animals were sacrificed 24hours after the end of treatments, the skin sample of the right paw was taken for TNF alpha evaluation and blood samples were obtained for assay of the levels of ALT, AST, ALP and C-reactive protein(CRP). Liver enzymes were determined using colorimetric methods while TNFalpha and CRP was determined using ELISA technique. Oral administration of the extract produced significant anti-edematogenic, anti-inflammatory activity and dramatic reduction in liver enzymes, TNF alpha and CRP with a dose of 150mg/kg and 300 mg/kg throughout the period of the experiment. This effect of *A.boonei* at 300mg/kg was similar to indomethacin group which showed a highly significant ( $p<0.05$ ) inhibition compared to control group. The result of this study suggests that aqueous extract of *A. boonei* possesses chronic anti-inflammatory activity which may be mediated by either inhibition or by blocking the release of prostaglandins, histamine and pro-inflammatory cytokine (TNF alpha), which suggest that *Alstonia boonei* is an alternative approach for treatment of chronic inflammatory disease, thus supporting its usage for anti- inflammatory treatment and potential effect to resuscitate the hepatocytes.

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**Keywords:** Anti-inflammatory, *Alstonia boonei*, Liver enzymes

### Introduction:

Inflammation is recognized as a vital process in response to the pathogenesis of various diseases, such as cardiovascular disease, cancer, atherosclerosis, arthritis, diabetes mellitus, obesity,

neurodegenerative disease, heart disease, and many other life-threatening and debilitating diseases. Inflammation is the activation of the immune system caused by infection, toxins, physical injury, or chemical irritation, and is a complex process

characterized by the contribution of several mediators, such as nitric oxide (NO), prostaglandins (PGs), tumour necrosis factor (TNF), interleukin-6 (IL-6), prostanoids, and leukotrienes<sup>1,2</sup>. Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly prescribed drugs for the treatment of inflammatory diseases<sup>3</sup>. NSAIDs possess analgesic and anti-inflammatory activity, because of the mechanism of inhibiting cyclooxygenases (COXs) for a decrease in PG production, which consequently reduces pain and inflammation. However, the NSAIDs, used clinically, are often of limited application, because of the occurrence of adverse digestive effects, most notably gastrointestinal haemorrhage, ulceration, and perforation<sup>4</sup>. Thus, developing a novel anti-inflammatory drug is crucial.

*Alstonia boonei*, a large deciduous tree belonging to the family Apocynaceae is one of the widely used medicinal plants in Africa and beyond which is known as *Awun* among the Yorubas of southwestern Nigeria, *Egbu-ora* among the Igbos of southeastern Nigeria and *Ukpukunu* by the Urhobos of south central Nigeria<sup>5</sup>. The important genus of the plants, *Alstonia* includes *A. scholaris*, *A. boonei*, *A. congensis* and *A. macrophylla* which have proved to be useful in various diseases. Almost all plant parts viz. leaves, stem bark; root bark and inflorescences have been used and are further under investigative study. It is distributed throughout the tropics and the rain forest of west and Central Africa<sup>6</sup>.

Various pharmacological studies have been carried out on this plant parts which showed that the extracts possess antimalarial, antipyretic, analgesic and anti-inflammatory properties, anthelmintic, diuretic, spasmolytic and hypotensive properties, Immuno-stimulant property, antipsychotic and anxiolytic effect, reversible antifertility effect<sup>6</sup>.

The liver is a complex organ with interdependent metabolic, excretory and defence functions. The use of several screening tests improves the detection of hepato-biliary abnormalities, helps differentiate the basis for clinically suspected disease and determine the severity of liver disease. Blood tests used for initial assessment of liver disease include measuring levels of serum Alanine and Aspartate aminotransferases (ALT and AST), Alkaline

phosphatase, and others. The pattern of abnormalities generally points to hepatocellular versus cholestatic liver disease and helps to decide whether the disease is acute or chronic and whether cirrhosis and hepatic failure are present. Serum enzyme level fluctuate widely from normal to moderately abnormal, with values rarely into the high hundreds. Marked elevation of aminotransferases in the appropriate clinical context indicates acute cell necrosis caused by viral infection, drugs, toxins, alcohol, or Ischemia<sup>7</sup>.

In this study, the anti-inflammatory activity of *A. boonei* aqueous stem bark extract and the effects of *A. boonei* aqueous stem bark extract on the liver enzymes of arthritic rats were evaluated using the formalin induced arthritis model, which was conducted in University of Benin, Benin- City, Edo State, Nigeria to know the anti-inflammatory effect. Since, nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of acute and chronic inflammation, pain and fever. But the greatest disadvantage in presently available synthetic drugs is that they cause gastrointestinal irritation and reappearance of symptoms after discontinuation. Therefore, there is a dire need for screening and development of novel, but better anti-inflammatory drug and indigenous medicinal plants which could be a logical source of solution to these limitations of nonsteroidal anti-inflammatory drugs (NSAIDs).

### **Materials and Method:**

#### **Plant Specimen Collection and Authentication**

The stem bark of *A. boonei* was collected from the growing tree inside University of Benin, Benin City in the month of June, 2020. It was identified and authenticated by Prof. Akinibosun A.O., Department of Plant Biology and Biotechnology, University of Benin, Benin City. The sample was washed, air dried and pulverized using plant milling machine (Kenwood UK LTD) the stem bark was stored in air tight containers prior to extraction.

#### **Aqueous extract preparation**

The powdered stem bark (400g) was boiled in 4L of distilled water for 15 minutes to obtain the aqueous extract. The extract was filtered and then concentrated under pressure in a rotary evaporator at 68°C and dried in an oven, at 50°C for 48 hrs

(yield 5.5%) the dried extract was stored in an air tight clean glass container at 4°C until use.

### Animals

Thirty (30) female wistar rats weighing between 150-230g were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Benin, Benin City. The animals were stabilized for two weeks in the animal house of the Department of Pharmacology, University of Benin, Benin City. The animals were fed with standard rodent cubes obtained from Ladokun feed Ltd. (Ibadan, Nigeria) and had free access to feed and water *ad libitum*. All animals were fasted overnight before the beginning of each experiment. Animals were exposed to natural lighting condition and were handled according to standard experimental protocols approved by the Faculty of Pharmacy Animal Ethics Committee, University of Benin, Benin City.

### Experimental design

Formalin induced arthritis inflammation

The animals were divided into 6 groups comprising of 5 animals in each group. They were fully described, weighed identified and marked, prior to the experiment.

Inflammation was induced by subaponeurotic injection of 0.1mL of 2% w/v formalin in normal saline in the right hind paw of the rats on the first and third day.

**Group 1:** This is the control group. This group received 3ml/kg of distilled water once a day for five days. They were not induced with 2% w/v formalin in normal saline. Neither plant extract nor indomethacin was administered to the animals in this group.

**Group 2:** This is the negative control group. This group received 3ml/kg of distilled water once a day for five days. They were induced with 2% w/v formalin in normal saline. Neither plant extract nor indomethacin was administered to the animals in this group.

**Group 3:** This test group received 150mg/kg, *p.o* of the extract once a day for five days.

**Group 4:** This test group received 300mg/kg, *p.o* of the extract once a day for five days.

**Group 5:** This test group received 600mg/kg, *p.o* of the extract once a day for five days.

**Group 6:** This is the reference group. This group received indomethacin (5mg/kg, *p.o.*) once a day for five days.

The rat paw thickness was measured daily for five days using Vernier caliper. The percentage inhibition of the mean increase in the paw oedema of each group was calculated on the 5<sup>th</sup> day and compared with the control. At the end of the experiment all the animals were anaesthetised using cotton wool soaked in chloroform. They were dissected using dissecting set. Blood was collected from abdominal aorta and directly from the heart using a 5mL syringe into a plain container and then allowed to clot. It was then centrifuged at 4000rpm for 10 minutes. The serum were collected into plain sterile containers and used for AST, ALT, ALP and CRP estimation. After wet- weigh measurement of paws, for TNF alpha evaluation, the skin sample of the right paw were homogenised in 3ml of phosphate buffered saline containing 10mmol/l EDTA and 20KIU/ml aprotonin (Sigma, MO, USA). After centrifuging at 10,000xg, the supernatant was frozen at -70<sup>0</sup>c for TNF alpha as described by previous report<sup>8</sup>.The levels of TNF alpha in paw supernatants were measured by ELISA assay specific for mouse TNF alpha(Santa Cruz Biotechnology, CA,USA)

### Statistical Analysis

The statistical analysis of the results were carried out. The various results obtained from this study were expressed as Mean ± Standard Error in Mean (S.E.M). The differences between the groups were determined by one-way ANOVA. The data were statistically analysed using SPSS Software version 23.0. The Tukey-Kramer Multiple comparisons Test was used as the post test for determination of significant difference between Means. A P-value (<0.05) was considered to be statistically significant and P-value (>0.05) was considered not statistically significant.

### Results;

Table 1 showed the effect of *A.boonei* aqueous extract on liver enzymes and anti-inflammatory marker on formalin –induced arthritic wistar rats. It was observed that the levels of liver enzymes and inflammatory markers (CRP and TNF alpha) were dramatically reduced on treatment with *A.boonei*

compared with group 2 (formalin – induced untreated group).

Table 2 showed the effect of *A.boonei* extract on formalin induced chronic inflammation in wistar rats. It was observed that aqueous extract of *A.boonei* at concentration of 600mg/kg and

300mg/kg have significant effect on reduction on paw thickness of wistar rat when compared with control group. Also the therapeutic efficacy of standard drug used (5mg/kg indomethacin) and 300mg/kg of *A.boonei* aqueous extract was similar ( $p<0.05$ ).

**Table 1: effect of aqueous extract of *a. Boonei* on formalin induced chronic inflammation in wistar rat**  
Value are expressed in mean±SEM.

Parameters	Control group	Group2	Group3	Group4	Group5	Group6	Pvalue
ALP(U/L)	76.04±2.80	96.66±2.68	92.20±1.60	64.40±1.70	82.20±1.20	50.41±1.60	P<0.05
AST(U/L)	73.40±5.02	207.60±1.60	195.16±4.60	78.96±1.20	100.16±1.40	40.12±1.50	P<0.05
ALT(U/L)	30.60±3.29	73.20±3.07	60.20±1.40	38.56±2.60	52.60±1.60	28.40±1.20	P<0.05
CRP(mg/L)	3.65±1.12	92.12±0.45	90.00±0.60	40.62±0.20	80.46±2.60	25.92±1.02	P<0.05
TNF@(pg/ml)	7.99±1.56	79.20±2.50	70.20±1.20	50.11±1.20	62.72±1.60	19.11±1.50	P<0.05

Group1- Control group, Group2-formalin induced group, Group3- 150mg/kg *A.boonei* extract, and Group4-300 mg/kg *A.boonei* extract group, Group 5-600mg/kg *A.boonei* extract group, Group 6- 5mg/kg Indomethacin group.

**TABLE 2: Effect of *Alstonia Boonei* Aqueous Extract on Liver Enzyme and Infla (Mmatory Markers on Formalin Induced Arthritic Wistar Rats**

Groups	Paw thickness at start-of treatment(mm)	Paw thickness at sacrifice(mm)	% Diff	%Inhibition
Control	8.55±0.09	8.65±0.11	0.10±0.02,1.2%	-
Formalin	8.65±0.26	11.62±0.30	2.97±0.04,34.3%	-
Indomethacin@5mg/kg	8.63±0.11	7.63±0.14**	1.00±0.03	11.59
<i>A.boonei</i> @600mg/kg	8.63±0.12	7.80±0.26*	0.83±0.1	9.60
<i>A.boonei</i> @300mg/kg	8.63±0.19	7.96±0.27**	1.01±0.06	11.70
<i>A.boonei</i> @150mg/kg	8.63±0.21	8.92±0.19++	0.29±0.08	3.30

### Discussion:

*Alstonia boonei* is a medicinal plant that has high therapeutic efficacy index as an antimalarial, antipyretic, analgesic, anti-inflammatory properties, anthelmintic, diuretic, spasmolytic and hypotensive properties, Immune-stimulant property, antipsychotic and anxiolytic effect, reversible antifertility effect<sup>6</sup>. This study was carried out to investigate the effect of *A. boonei* aqueous stem bark extract on the serum levels of ALP, AST, ALT and CRP in formalin induced arthritic rats. In the present study, the anti-inflammatory activity of the aqueous extract of *A. boonei* has been evaluated in chronic inflammatory model. The inhibition of formalin induced inflammation in rats is an

established model for evaluating anti-inflammatory drugs, which has been used frequently to assess anti-oedematous effect of natural products. Formalin injection causes a chronic inflammation of the rat foot, which involves the proliferation phase of inflammation elicited by COX mediators and pro-inflammatory cytokine. Then, it is logical that indomethacin, an NSAIDs that inhibits COX-2 enzyme isoform, effectively reduce the formalin-induced increases in the size of the rat paw. TNF alpha is key pro-inflammatory cytokine implicated in acute and chronic inflammation<sup>8,9,10</sup>. NSAIDs being the mostly used drugs for treatment of inflammatory diseases has some limitations like gastrointestinal irritation(indomethacin), hence the

study of the medicinal plant (*A. boonei*) which has showed remarkable therapeutic effect on inflammatory diseases. The finding was in agreement with Olajide *et al.*, (2000)<sup>11</sup> who reported that methanol extract of *A. boonei* caused a significant inhibition of the carrageenan-induced paw oedema (paw thickness), cotton pellet granuloma, and exhibited an anti-arthritic activity in rats induced with formalin. It was recently reported that TNF alpha has a role of triggering others pro-inflammatory substance<sup>12</sup>. In this study, *A. boonei* dose dependent treatment has inhibited the elevation of TNF alpha and CRP induced in wistar rats by subaponeurotic injection of formalin. Therefore, we considered that *A. boonei* extract showed favourable anti-inflammatory effect on chronic inflammation mediated by cytokine TNF alpha suppression and resuscitation of tissue damage to the liver. However, it should be put to note that the immunomodulatory, antioxidative and anti-hyperlipidemic effect of *A. boonei* may also have been implicated in the anti-inflammatory activity observed in the present, because inhibition of nitric oxide synthases can reversed classic inflammatory symptoms, and immunomodulatory agent can reduce inflammation<sup>13,14</sup>.

Serum or plasma liver enzyme levels are considered as markers for monitoring the degree of chemically induced liver damage<sup>15,16,17</sup>. The plasma activity of ALP, AST and ALT determines the functionality of the liver<sup>18</sup>. ALT and AST are biomarkers of the hepatocytes; they are intracellular enzymes necessary for amino acid production<sup>19</sup>. Under pathological conditions of the liver including cirrhosis, acute hepatitis, osteohepatitis, and adverse effects of some drugs (e.g. paracetamol), there is a leak of the enzymes into the plasma, thus raising their activity<sup>20,21</sup>. ALT is specific for the liver but AST is also found in other tissues including the red blood cells, the cardiac and the skeletal muscles. The increased level of serum ALT, AST, ALP and CRP following subaponeurotic injection of formalin indicate the level of chronic inflammation and tissue damage that has occurred. The oral administration of the plant extract after inducing the rats with 2% w/v formalin in normal saline at the doses of 150mg/kg and 300mg/kg body weight (group 3 and 4) respectively has ameliorate the

hepatotoxicity associated with the formalin at these doses<sup>22</sup>.

There was a highly significant reduction in serum AST and ALT concentration ( $p < 0.05$ ) in group 6 (Reference group) when compared to the negative control group which suggest the anti-inflammatory therapeutic efficacy of *A. boonei* at dosage of 300mg/kg compared with 5mg/kg indomethacin was similar, therefore, *A. boonei* extract may present an alternative approach for the treatment of chronic inflammatory disease with relatively fewer side effect as seen with NSAIDs (indomethacin). Also observed that increase in dosage of *A. boonei* extract can induced hepatotoxicity whose underlying mechanism is not unclear, but further research will elucidate the effect.

Serum alkaline phosphatase (ALP) is a sensitive detector in biliary cirrhosis, hepatitis and in diseases characterized by inflammation, regeneration, intrahepatic and extrahepatic bile obstruction<sup>23,24</sup>. The highly significant dramatic reduction of ALP seen at group 3, group 4 showed that the plant extract has the potential to resuscitate the hepatocytes from damage

### **Conclusion:**

In conclusion, the aqueous extract of *A. boonei* has been shown to be effective against chronic inflammation (formalin induced paw oedema) in a dose related manner. This present study supports the claim in the use of the extract of *A. boonei* in traditional medicine for the treatment of inflammatory conditions and it has also shown that the aqueous extract of *A. boonei* has the potential to restore the cellular integrity and functionality of the liver. Based on the findings, we conclude that *A. boonei* treatment had a favourable effect by TNF alpha suppression on reducing the inflammatory activity.

### **Conflict of interests**

The authors have not declared any conflict of interest.

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