



Toxicological Assessment of Synergistic Efficacy of *Alstonia boonei* & *Capacium frutescens* Extract on *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice

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Abstract

Malaria/Typhoid is one of the most killing diseases in the world particularly in tropical countries and is worst in Africa. The study was conducted to determine the anti-malaria/anti-typhoid potentials of graded doses coupled with the toxicological and histopathological effect of synergistic aqueous and ethanolic extract of *Alstonia boonei* stem bark (Epo ahun) and fruit of *Capsicum frutescens* in Swiss albino mice infected with *Plasmodium berghei* NK 65 and *Salmonella typhi* (ATCC 35723). The stem bark and fruit of *Alstonia boonei* and fruit of *Capsicum frutescens* were screened for the presence of some phytochemicals. Twenty five Swiss Albino mice were divided into 5 groups of 5 mice each. The animals were inoculated with the parasite and *Salmonella typhi* (ATCC 35723) at the beginning of the experiment (day 10). Three hours after inoculation (infestation 0 groups 1-3) mice were respectively given 100 mg, 200 mg and 400 mg combined extract/kg body weight dose intraperitoneally. Group 4 animals which serve as control were given 5 mg Chloroquine/kg body weight while the group 5 animals (negative control) were given 0.2 ml distilled water. The treatment was given ones per day for four days. On the 5th day, the animal caudal and vein samples were taken and transferred into a slide making thin film from each mouse. The percentage chemo-suppressive activity on early malaria and typhoid infection in mice of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight dose were found to be 81, 85, 75 respectively for ethanolic extract and 57%, 78% and 80% respectively for aqueous extract this is substantial when compared to 97% chemosuppressive effect produced by 5 mg/kg body weight of Chloroquine/Ciprofloxacin. The phytochemical screening of the combined extract reveals the presence of Saponins, Flavonoids, Terpenes, Alkaloids, Glycosides, Terpenoids while anthroquinones and acid compounds were found absent. The acute toxicity (LD₅₀) of the combined extract was estimated to be 3162 mg kg⁻¹ b.wt. The above result showed that the combined extract of stem bark of *Alstonia boonei* and fruit of *Capsicum frutescens* possesses antiplasmodial and anti-typhoid property. The toxicology and histopathological study of the synergistic extract of *Alstonia boonei* and Fruit of *Capsicum frutescens* were studied viewing the liver enzymes and kidney function on slide after staining. The results from this findings shows an increase in the serum Alanine Aminotransaminase (ALT), Aspartate Aminotransaminase (AST), Alkaline Phosphatase (ALP), Bilirubin, Blood Urea Nitrogen (BUN) and Creatinine level of the liver and kidney of the infected Swiss Albino Mice. This dose dependent increase is an indication of toxicity of the extract which calls for a moderate use of the extract.

Keywords: Anti-Malaria/Anti-Typhoid; Toxicological assessment; Synergistic extract *alstonia boonei*; *Capsicum frutescens*; Swiss albino mice

Introduction

Alstonia boonei, among other plants, has been noted as a good medicinal plant for curing various diseases. *Alstonia boonei* which belongs to the Apocynaceae family have severally been reported to have medicinal properties. As such, they are used by traditional health practitioners especially in rural areas. Several species of *Alstonia boonei* abound including *Alstonia macropylla*, *Alstonia scholaris* etc. But this species are not as popular as *Alstonia boonei*, which have been widely reported in Nigeria. Typically, *Alstonia boonei* is a facultative plant having estimated occurrence probability of 33% to 67% in both wetland and non-wetland areas. Also *Alstonia boonei* possesses therapeutic properties probably due to the presence of bioactive constituents and metabolites.

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Received Date: 27 Mar 2020

Accepted Date: 15 Apr 2020

Published Date: 24 Apr 2020

Citation:

Osuntokun OT, Ajiga PJ. Toxicological Assessment of Synergistic Efficacy of *Alstonia boonei* & *Capacium frutescens* Extract on *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice. *Ann Pharmacol Pharm.* 2020; 5(4): 1187.

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Studies have shown that *Alstonia boonei* have several medicinal properties both for human and other mammals. For instance studies has indicated that a *boonei* have Antihyperglycemic and antioxidant, wood healing properties [1-3]. Others include Antiplasmodial activities against *Plasmodium berghei* infection in mice [4], Analgesic effects [5], enhancement of rotarod period in albino mice, diuretic properties in male Wistar rats [6], treatment of chronic Diarrhea and dysentery, fever, pain, intestinal disorders and as an antidote for *Strophanthus* poison [7]. Anti-snake venom and as antidote to some arrows poisons [8], treatment of malaria, typhoid fever, gonorrhoea, yaws, asthma, dysentery, and as a galactagogue [9], and antimicrobial properties different tissues extract of *Alstonia boonei* have been severally reported to contain some essential phytochemicals [10].

Alstonia boonei comprises about 40 species and has a pantropical distribution. There are about twelve species of the genus *Alstonia*. *Alstonia boonei* wild belongs to the family Apocynaceae. The species are scattered all over the world of which two are indigenous to Africa. The plant is known locally in Ghana as Onyame dua, Osenuru, or Sinduro in twi, onyame dua in fante, sinu or adawura in ga-adangbe, bakunin, nyamenlebaka, emenle, or Emie in nzema, and Siaketekre, Nyemi dua, or asi atoe in ewe [11].

Therapeutically, the stem bark has been found to possess ant rheumatic, anti-inflammatory, analgesic/pain-killing, antimalaria/antipyretic, antidiabetic (mild hypoglycaemic), antihelminthic, antimicrobial and antibiotic properties [12]. *Alstonia boonei* decoction also exerts a mild antibacterial effect in this case, relieving the aches and pains associated with malaria fever. *Alstonia boonei* is taken in the form of preparations that exhibits ant pyrexia and anti-malaria effects, to combat rheumatic and arthritic pains. The decoction of *Alstonia boonei* bark could be taken alone as an effective pain-killing agent. A cold infusion made from the fresh or dried bark of *Alstonia boonei* taken orally two to three times daily exerts a mild hypoglycemic effect on diabetic patients. *Alstonia boonei* De Wild is regarded as one of few herbs with potential anti-HIV indicators. In some African countries *Alstonia boonei* is considered a sacred tree and worshiped in the forest and hence human beings in those countries do not eat its parts [12].

Capsicum frutescens is a species of chili pepper that is sometimes considered to be part of the species *K. pepper* cultivars of *Capsicum frutescens* can be annual or short-lived perennial plants. Flowers are white with a greenish white or greenish yellow corolla, and are either insect- or self-pollinated. The plants' berries typically grow erect; ellipsoid-conical to lanceoloid shaped. They are usually very small and pungent, growing 10 mm to 20 mm (0.39-0.79 in) long and 3 mm to 7 mm (0.12-0.28 in) in diameter. (Y) Fruit typically grows a pale yellow and matures to a bright red, but can also be other colors. *Capsicum frutescens* has a smaller variety of shapes compared to other capsicum species. *Capsicum frutescens* has been bred to produce ornamental strains, because of its large quantities of erect peppers growing in colorful ripening patterns.

The prevalence of malaria/typhoid fever as well as growing incidence of the death resulting from the disease coupled with the increase in the resistance of malaria parasite and *Salmonella typhoid* to synthetic drugs has led to the increase search for alternative treatment strategy [12].

Plants are cheap sources of medicinal intervention both for curative and preventive measures in Africa and Asia continents, a greater percentage of the world population solely depend on

medicinal plants; hence need to carry out researches on the particular dosage needed to suppress the ailment. The synergistic ethanolic extract of *Alstonia boonei* and fruit of *Capsicum frutescens* has been scientifically screened to contain certain phytochemicals which are responsible for the prevention or treatment of malaria parasite and *Salmonella typhi* and with acute toxicity which may be unknown to some individuals. The present study is thus necessary to fill the lacuna as it establishes the curative power and toxic effect of the synergistic extract on the treatment malaria and typhoid [13].

Materials and Methods

Apparatus

The apparatus used include beaker, conical flasks, measuring cylinders, weighing balance, universal centrifuge and volumetric flasks. Thermometer, glass pipette, syringes and needle, test tubes and racks, spatula, glass rod, reagent bottles, water bath, UV-visible spectrophotometer, dissecting board, dissecting set, sample bottles, funnel, oral intubator (cannular), PH meter, microscope, gloves, -20°C and -80°C refrigerator, kidney function and liver function kits, petri-dishes.

Reagents

Washing buffer (1.15% chloride) 1.15 g of potassium chloride (BDH chemical limited, England) was dissolved in 100 ml distilled water and made up to 1000 ml and stored at 4°C homogenizing buffer (0.1 m Phosphate buffer, PH. 7.4).

(a) 11.8 g of Na_2HPO_4 (sigma chemical Co. St. Louis U.S.A.) was dissolved in 1000 ml distilled water.

(b) 6.8 g of KH_2HPO_4 was dissolved in 500 ml distilled water, then 800 ml's of (a) was mixed with 200 ml of (b) above to make 1000 ml. The P^{H} was adjusted to 7.4 with 1M NAOH. This was then stored at 4°C till use.

Plant identification

The *Alstonia boonei* and *Capsicum frutescens* were identified based on the description provided by Dr. Obembe of Plant Science and Biotechnology Department, Adekunle Ajasin University Akungba Akoko. Some of the description includes a large deciduous evergreen tree that could reach 45 m tall and 1.2 m in diameter, deeply fluted to 7 m, small buttresses grayish-green or grey for the bark, milky latex from the leave and bark and 5-7 whorls of leaves (Figure 1,2). The plant has several English names including stool wood, Cheesewood, pattern wood, *Alstonia boonei*.

Collections of samples

The stem bark of *Alstonia boonei* and *Capsicum frutescens* used in this study was collected early in the morning from within Adekunle Ajasin University, Akungba Akoko, Nigeria premises.

Sample preparations

The collected stem bark of *Alstonia boonei* and *Capsicum frutescens* was washed with water and screened for foreign object and to get rid of dirt after which it was air dried for about four weeks and ground into fine powder under aseptic conditions. Using a mechanical grinder; about 1000 g of *Alstonia boonei* and 300 g of *Capsicum frutescens* powder was weighed into 500 ml of (80% ethanol and 25% water) in a bottle which was covered. The solution was filtered using Whatman filter paper. The filtrates were evaporated to dryness using a rotary evaporator and stored at 4°C until required for use [13,14].

Parasites used

The *Plasmodium berghei* was obtained from Institute of Medical Research and Training (IAMRAT) College of Medicine, University of Ibadan, Oyo State, Nigeria. A standard inoculum of 1×10^7 of parasitized erythrocytes from a donor mouse in volume of 0.2 ml was used to infect the experimental animals intra-peritoneally.

Organism used

Salmonella typhi (ATCC 35723) was used for this research work. It was obtained from (IAMRAT) College of Medicine, University of Ibadan, Oyo State, Nigeria. A standard inoculum of 2×10^8 acetone-killed *Salmonella typhi* Ty2 with the-vi antigen-free variant 0-901 was used to infect the Swiss Albino mice intra-peritoneally.

Experimental animal

Swiss albino mice between 15 g to 20 g obtained from animal house, IAMRAT, College of Medicine, and University of Ibadan were used for the experiment.

Acute toxicity studies (LD₅₀)

The median Lethal Dose (LD₅₀) of the synergistic extract of *Alstonia boonie* stem bark and *Capsicum frutescens* fruit at 60/40 percentile that will kill 50% of the animals in a population was determined intra-peritoneally using the method described by Alaribe et al. [15] (Figure 3,4). The mice were divided into five groups of four mice each weighing between 13g to 23 g. The mice were subjected to 24 h fasting (with only water) before administration of the synergistic extracts. The extract was dissolved in 20% Tween-80 and administered in doses of 500 mg kg⁻¹, 1,000 mg kg⁻¹, 2,000 mg kg⁻¹, 3,000 mg kg⁻¹ and 4,000 mg kg⁻¹ b.wt intra-peritoneally. The sixth group served as the control and received only 20% Tween-80. The mice were then observed for toxicity and fatalities over 72 h (40,15).

The LD₅₀ was calculated using the equation of Lorke (1983):

Where: Square root of A and B

a= Least tolerable dose

b= Maximum tolerable dose

Administration of extracts

The mice parasitized with *Plasmodium berghei* (NK 65) and *Salmonella typhi* (ATCC 35723) were sacrificed after six days, having been observed to have shown clinical symptoms of malaria and typhoid fever. The mice were anaesthetized in a glass jar containing cotton wool soaked in Chloroform. Blood was collected from the sacrificed mice by cardiac puncture using sterile syringes and needles. The blood was diluted in normal saline in the ratio of 1:10 (1 mL of blood in 10 mL of normal saline). The organism and parasitized Erythrocyte in volume of 0.3 mL was used to infect each of the experimental mice intra-peritoneally six days before treatment [16].

Test for anti-malaria/anti-typhoid activity: The suppressive antimalarial test also called "Test on Early Malaria Infection" as reported by Majekodunmi was used in this study [17]. For the Ethanolic and aqueous extracts (separately), 25 mice were divided into 5 groups of five mice each. The animals were inoculated with the parasite/organism at the beginning of the experiment (day 10). Three hours after inoculation (infestation 0 groups 1-3 mice were respectively given 100 mg, 200 mg, and 400 mg extract/kg body weight dose orally, group 4 animals, which served as positive control were given 5 mg chloroquine/Ciprofloxacin kg body weight while

the group five animals (negative control) were given 0.2 ml distilled water. The treatment was given once per day for four days. On the 5th day, two drops of the animals caudal vein blood samples were taken and transferred on slides making thin film from each mouse. The slides were stained with Giemsa stain and subjected to microscopy. The average percentage parasitaemia/typhoidal and hence percentage chemo-suppression were evaluated for each of the does using the formulae:

$$\% \text{ Suppression} = \frac{\text{Average parasitaemia in negative control} - \text{Average parasitaemia/typhoidal (in test dose)}}{\text{Average parasitaemia/typhoidal in negative control}} \times 100 \text{ [17].}$$

Tissue histopathology: The fixed liver and kidney tissues were dehydrated by ascending grades of isopropyl alcohol for an hour. The dehydrated organs were cleared in xylene and transferred into two changes of liquid paraffin wax. The tissue sections were stained in Ehrlich's hematoxylin for 8 min, washed in water and dipped in acid alcohol to remove excess stain. These were counter stained in 10% aqueous eosin, incubated and mounted for photomicrography [18].

Phytochemical screening of *Alstonia boonie* and *Capsicum frutescens*: The secondary metabolite (phytochemical) screening of the sample was carried out as described by [18]. The samples were screened for the following components.

- **Test for saponins:** To 1 ml of plant extract, 5 ml to 10 ml of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise. Formation of 1cm layer of foam indicates the presence of Saponins.
- **Test for flavonoids:** A fraction of the extract was treated with concentrated sulphuric acid and observed for the formation of orange color.
- **Test for alkaloids:** To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates alkaloids.
- **Test for glycosides:** To 2 ml of plant extract, 1 ml of glacial acetic acid and 5% ferric chloride was added. Then few drops of concentrated sulphuric acid were added. Presence of greenish blue color indicates glycosides.
- **Test for terpenoids and steroids:** A fraction of the extract was dissolved in chloroform. A few drops of acetic anhydride was added followed by two drops of conc. H₂SO₄. Reddish-pink coloration indicates terpenoids and steroids.
- **Test for carotenoids:** To about 2 ml of the extract, 3 ml of antimony trichloride was added. Dark-blue coloration is indicative of carotenoids.
- **Test for coumarins:** A small quantity of plant extract was taken into a test-tube. The test-tube was then covered with a piece of filter paper moistened with dil. NaOH solution and placed in a hot water bath. After about 15 min, the paper was removed and exposed to U.V light. Yellow-green fluorescence indicates the presence of coumarins.
- **Test for anthraquinones:** A small amount of the extract was boiled with 25 ml of 0.5M KOH and 4 ml of H₂O₂. The mixture was then cooled and acidifies with a few drops of acetic acid. The acidulated mixture was extracted with a small amount of benzene (15

Table 1&2: The qualitative secondary metabolite (phytochemical screening) of *Alstonia boonie* (stem bark) & *Capsicum frutescens* (fruit).

Phytochemical constituents analyzed	<i>Alstonia boonie</i> (stem bark)	<i>Capsicum frutescens</i> (fruit)
Alkaloids	+ve	+ve
Tannins	+ve	+ve
Saponins	+ve	+ve
Anthroquinones	-ve	-ve
Steroids	+ve	+ve
Terpenoids	+ve	+ve
Flavonoids	+ve	+ve
Glycosides	+ve	-ve
Acid compounds	-ve	-ve

Key: +present, -absent

Table 3: Effect of ethanolic extract of *Alstonia boonie* stem bark (60%) and *Capsicum frutescens* (40%) on early malaria/typhoid infection in Swiss Albino Mice.

Treatment	Dose concentration (mg/kg/day)	Average suppression*	Percentage suppression (%)
Extract- <i>Alstbo</i> stb (60%) & <i>Capfr</i> (40%)	100	10.20 ± 0.86	81
Extract- <i>Alstbo</i> stb (60%) & <i>Capfr</i> (40%)	200	8.00 ± 0.77	85
Extract- <i>Alstbo</i> stb (60%) & <i>Capfr</i> (40%)	400	13.75 ± 3.12	75
Chloroquine/ Ciprofloxacin	5	1.60 ± 0.24	97
Distilled water	0.2 ml	54.40 ± 4.45	0

Key Extract: *Alstonia boonie* stem bark (60%) and *Capsicum frutescens* (40%)

ml). The benzene extract was shaken with a small amount of NH_4OH . Red coloration indicates anthraquinones. Colorless alkaline layer indicates absence of anthraquinones.

- **Test for anthraquinone glycosides:** To about 2 ml of the extract, 20 ml of dilute H_2SO_4 was added and boiled. The mixture was filtered hot and a portion of the cooled filtrate was shaken with an equal volume of benzene. The benzene layer was separated and shaken with about half its volume of dilute NH_3 solution. A colorless ammoniacal layer indicates the absence of anthraquinone glycosides.

- **Test for cyanogenetic glycosides:** To about 2 ml of the extract was taken into a test-tube. A few drops of chloroform was then added and a piece of moist sodium picrate paper was inserted into the test-tube, taking care that it does not come into contact is kept warmed at 35°C for about 3 h. The presence of red color of the sodium picrate paper after the 3 h indicates cyanogenetic glycosides.

Biochemical assay: Serum biochemical parameters Alanine Transaminase (ALT), Aspartate Transaminase (AST) Alanine Phosphates (ALP), Bilirubin, Blood urea Nitrogen and Creatinine were estimated using commercial kit. Preparation of tissue homogenate and blood collection for biochemical analyses. The animals were sacrificed on the 24 hours after the administration of the last treatment, i.e. Second day; three rats were sacrificed from each group while blood samples, liver and kidney were obtained for biochemical analysis and histopathology. The blood samples were then centrifuged for 10 minutes at 3,000 g using bench centrifuge. The supernatant (serum) was collected and serum Alanine Aminotransaminase (ALT), Aspartate Aminotransaminase (AST), Alkaline Phosphatase (ALP), Bilirubin, Blood Urea Nitrogen (BUN) and Creatinine were determined [19].

Statistical analysis: The results were expressed in terms of mean

Table 4: Effect of aqueous extract of *Alstonia boonie* stem bark (60%) and *Capsicum frutescens* (40%) on Malaria/Typhoid infected Swiss Albino mice.

Treatment	Dose concentration (mg/kg/day)	Average suppression*	Percentage suppression
<i>Alstbo</i> stb (60%) & <i>Capfr</i> (40%)	100	25.00 ± 0.5	56
<i>Alstbo</i> stb (60%) & <i>Capfr</i> (40%)	200	12.20 ± 2.25	78
<i>Alstbo</i> stb (60%) & <i>Capfr</i> (40%)	400	11.50 ± 2.72	80
Chloroquine/ Ciprofloxacin	5	1.60 ± 0.24	97
Distilled water	0.2	54.40 ± 4.45	0

± standard deviation (sd). Parameters in the groups were compared by one-way (anova) using spss version 15 significant differences were set at ($p < 0.05$).

Result and Discussion

The results of the secondary metabolite (phytochemical screening) of stem bark of *Alstonia boonie* showing the presence of Alkaloids, Tannins, Saponins, Steroids, Flavonoids and Cardiac Glycosides in substantial quantities (Table 1). Anthroquinones and acid compounds were however, not found in the plant stem bark. The presence of alkaloids in high concentration in the plant stem bark explains the traditional use of the plant for the treatment of malaria. The medicinal plants that are moderately rich in alkaloids and tannins have potential health promoting effects.

Table 2 showing the results of the phytochemical screening of *Capsicum frutescens*, it shows the presence of Alkaloids, Tannins Saponin, steroids, flavonoids, Carotenoids, Terpenoids, while Anthroquinones, Glycosides and Acid compounds were absent.

Table 3 shows the effects of ethanolic extract of *Alstonia boonei* stem bark powder on early infection in mice. The *In-vivo* evaluation revealed that the average percentage suppression of parasitaemia/typhoid by the extract was 81%, 85% and 75% at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight per day, respectively.

On the other hand, portrays the effect of the aqueous extract on the same early malaria/typhoid infection (Table 4). From the table, the average percentage suppression of parasitaemia/typhoidal was 56%, 78% and 80% at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg/day respectively. Chloroquine/Ciprofloxacin, under the same experimental condition, at 5 mg/kg body weight per day produced chemo-suppression of 97%.

The effect of synergistic acute administration of the synergistic extract *Astonica boonie* and *Capsicum frutescens* at 60/40 on liver

Table 5: Showing effect of acute administration of *Astonia boonie* (60%) and *Capsicum frutescens* (40%) on liver enzymes.

Group	Dose Concentration	AST(u/l)	ALT(u/l)	ALP(u/l)
1	Distilled water 10ml/kg	43.45 ± 0.3	43.45 ± 0.4	29.11 ± 2.1
2	Synergistic extract (<i>Alstbo</i> stb (60%) & <i>Capfr</i> (40%) 1000 mg/kg	46.07 ± 0.1	57.52 ± 1.2	33.02 ± 1.2
3	Synergistic extract (<i>Alstbo</i> stb(60%) & <i>Capfr</i> (40%) 2000 mg/kg	131.02 ± 1.1	140.44 ± 0.1	84.21 ± 0.3
4	Synergistic extract <i>Alstbo</i> stb (60%) & <i>Capfr</i> (40%)4000 mg/kg	86.90 ± 0.21	86.90 ± 1.3	67.32 ± 2.1

Key Extract: *Alstonia boonie* stem bark (60%) and *Capsicum frutescens* (40%)

* $p < 0.05$ = Significant when compared with distilled water, where (AST) means Assay of Aspartate Aminotransferase; ALT: Assay of alanine amino transferase, Alkaline phosphate

Table 6: showing effect of acute administration of *Alstonia boonie* and *Capsicum frutescens* on kidney function test.

Group	Dose Concentration	Bilirubin (mg/dl)	Bun (mg/dl)	Crt (mg/dl)
1	Distilled water 10 ml/kg	1.95 ± 1.2	11.90 ± 0.3	1.80 ± 0.1
2	Synergistic extract (<i>Alstbo stb</i> (60%) & <i>Capfr</i> (40%) 1000 mg/kg	2.16 ± 0.2	12.45 ± 0.4	1.80 ± 2.2
3	Synergistic extract (<i>Alstbo stb</i> (60%) & <i>Capfr</i> (40%) 2000 mg/kg	3.50 ± 1.1	12.50 ± 1.3	2.00 ± 1.2
4	Synergistic extract <i>Alstbo stb</i> (60%) & <i>Capfr</i> (40%) 4000 mg/kg	2.50 ± 0.1	12.86 ± 1.0	2.20 ± 0.3

BUN: Blood Urea Nitrogen; CRT: Creatinine



Figure 1: Plate showing clockwise order from top right, the leaves, stem, and branches of *Alstonia boonie*.



Figure 2: Picture of *Capsicum frutescens*.

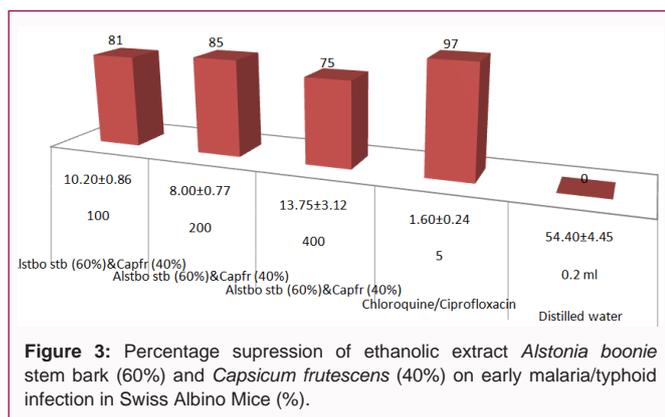


Figure 3: Percentage suppression of ethanolic extract *Alstonia boonie* stem bark (60%) and *Capsicum frutescens* (40%) on early malaria/typhoid infection in Swiss Albino Mice (%).

enzymes which include the Alanine Transaminase (ALT), Aspartate Transaminase (AST) Alanine Phosphates (ALP) (Table 5). Distilled water of 10 ml/kg was used as a control on the liver enzymes, after which a dose of the synergistic extract ranging from (1000, 2000, and 4000) mg/kg is used. The result shows a high variation when 2000 mg/kg of the synergistic extract was used which later reduce at 4000 mg/kg dose.

The histological photograph of the liver enzymes taken at each dose was stained and viewed under microscope (He x400):

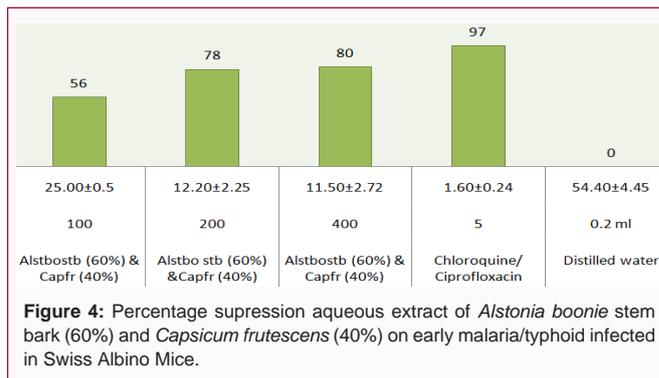


Figure 4: Percentage suppression aqueous extract of *Alstonia boonie* stem bark (60%) and *Capsicum frutescens* (40%) on early malaria/typhoid infected in Swiss Albino Mice.

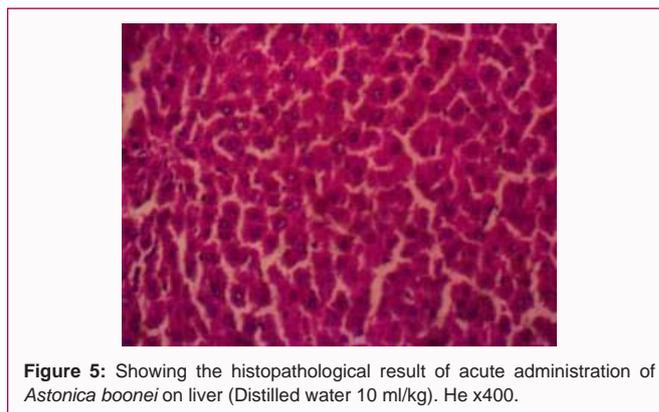


Figure 5: Showing the histopathological result of acute administration of *Alstonia boonei* on liver (Distilled water 10 ml/kg). He x400.

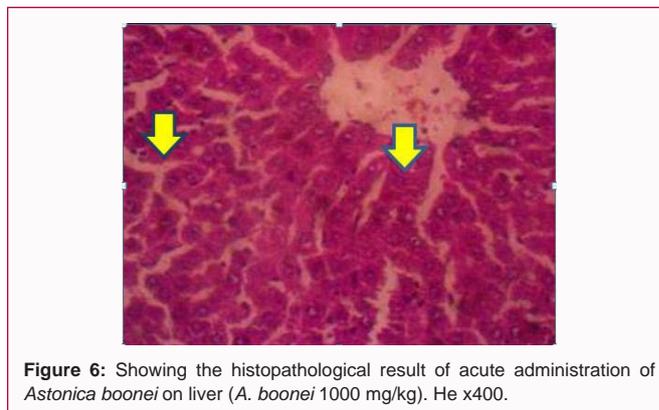


Figure 6: Showing the histopathological result of acute administration of *Alstonia boonei* on liver (*A. boonei* 1000 mg/kg). He x400.

Group 1: Distilled water (10 ml/kg) - There is no observable lesion (Figure 5).

Group 2: Synergistic extract (1000 mg/kg) - There is moderate atrophy of hepatic cords and accentuation of sinusoids (arrows) (Figure 6).

Group 3: Synergistic extract (2000 mg/kg) - There is centrilobular hepatocellular generation, necrosis (blue arrow) and inflammation (black arrow) (Figure 7).

Group 4: Synergistic extract (4000 mg/kg) - There is centrilobular hepatocellular generation, necrosis (blue arrow) and inflammation (black arrow) (Figure 8).

The effect of acute administration of synergistic extract of *Alstonia boonie* and *Capsicum frutescens* at 60/40 on kidney function test (Table 6). A group of Swiss albino mice were treated with distilled water as control experiment. The kidney functional test was carried out on the Bilirubin, Blood Urea Nitrogen and Creatinine. There is

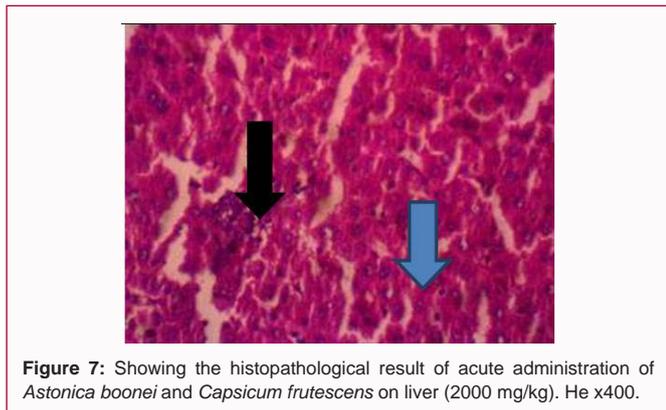


Figure 7: Showing the histopathological result of acute administration of *Alstonia boonei* and *Capsicum frutescens* on liver (2000 mg/kg). He x400.

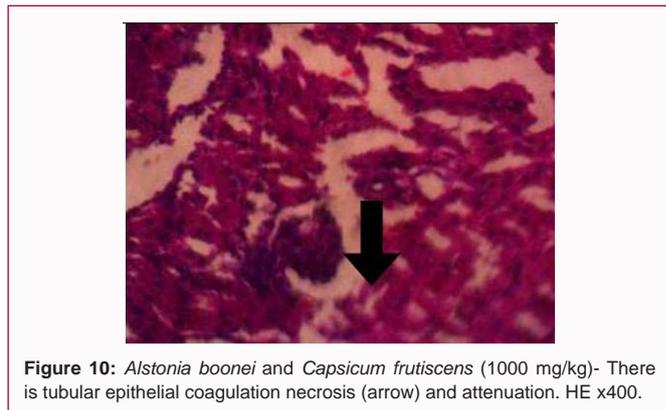


Figure 10: *Alstonia boonei* and *Capsicum frutescens* (1000 mg/kg)- There is tubular epithelial coagulation necrosis (arrow) and attenuation. HE x400.

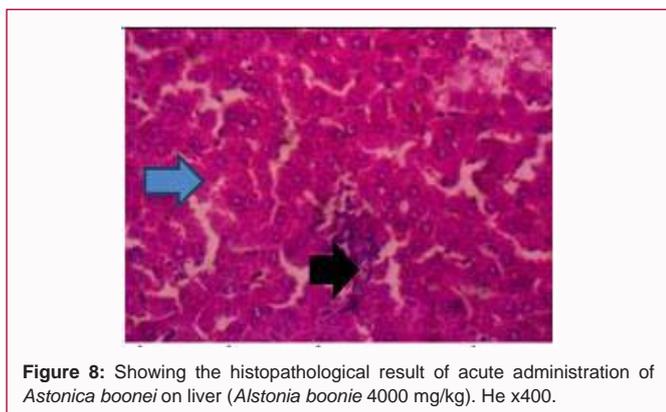


Figure 8: Showing the histopathological result of acute administration of *Alstonia boonei* on liver (*Alstonia boonei* 4000 mg/kg). He x400.

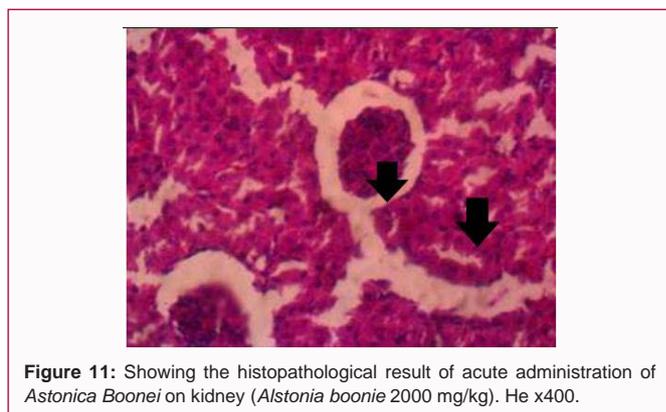


Figure 11: Showing the histopathological result of acute administration of *Alstonia Boonei* on kidney (*Alstonia boonei* 2000 mg/kg). He x400.

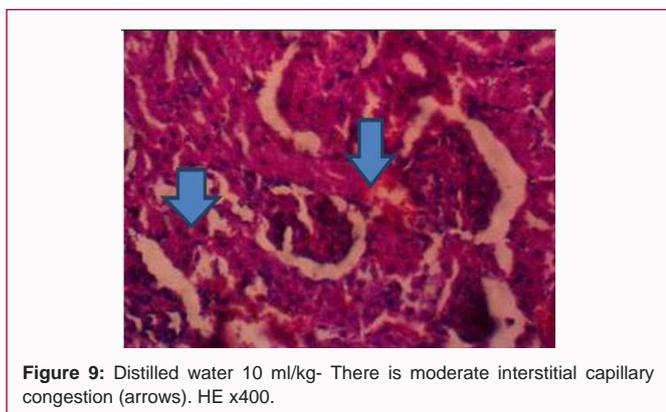


Figure 9: Distilled water 10 ml/kg- There is moderate interstitial capillary congestion (arrows). HE x400.

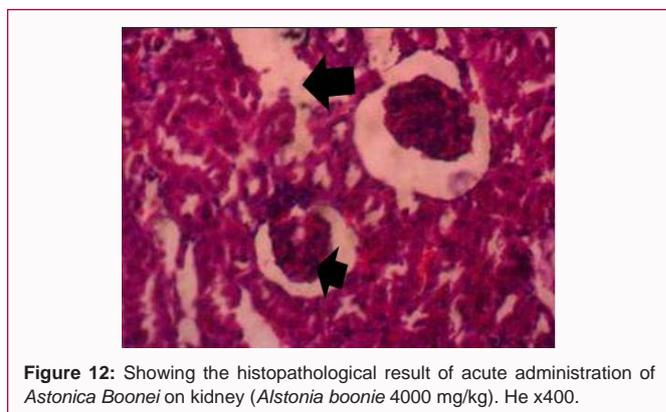


Figure 12: Showing the histopathological result of acute administration of *Alstonia Boonei* on kidney (*Alstonia boonei* 4000 mg/kg). He x400.

a noticeable increase in the kidney activity on administration of the synergistic Ethanolic extract when compared to the kidney function when extracts are not utilized.

The histological photograph of the kidney function taken at each dose was stained and viewed under microscope (He x400).

Group 1: Distilled water (10 ml/kg) - There is moderate interstitial capillary congestion (arrows) (Figure 9).

Group 2: Synergistic extract (1000 mg/kg) - There is tubular epithelial coagulation necrosis (arrow) and attenuation (Figure 10).

Group 3: Synergistic extract (2000 mg/kg) - There is patchy tubular epithelial coagulation necrosis (arrows) and attenuation (Figure 11).

Group 4: Synergistic extract (4000 mg/kg) - There is tubular epithelial coagulation necrosis (arrows) and luminal Ectasias (Figure

12).

Discussion

This study the toxicological assessment of synergistic extract *Alstonia boonei* & *Capacium frutescens* extract on plasmodium berghei (NK 65)/*Salmonella typhi* (ATCC 35723) infected Swiss albino mice it also evaluates the antimalarial/anti-typhoid activity of ethanolic and aqueous stem bark of *Alstonia boonei* and *Capsicum frutescens* coupled with toxicological effect on the liver and kidney of Swiss Albino Mice.

Currently no single drug was effective for treating multi-drug resistant malaria/typhoid, effective combination therapy includes Artemisinin derivatives such as Artesunate, or mixtures with older drugs such as the atovaquone, Proguanil, combination Malarone [19-21]. Unfortunately first reports on drug resistance to Artemisinin-derivatives and to drug combination therapies have already appeared

[22,23]. This shows a greater need to synergize two or more plants with different phytochemical constituents to cure diseases.

In the absence of a functional, safe and widely available malaria/typhoid vaccine, efforts to develop new antimalarial/anti-typhoid drugs continue. There has been a consensus among the scientific community that natural products have been playing a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases [24]. Indeed, the vast majority of the existing anti-malarial/anti-typhoid chemotherapeutic agents are based on natural products and this fact anticipates that new leads may certainly emerge from the tropical plant sources, since; biological chemo-diversity continues to be an important source of molecular templates in the search for antimalarial drugs [25]. More so, there is need to consider what to use for the extraction of the plants as this too may have a positive effect on the biochemical reaction of the drug at certain dose administered by the animal. The liver and kidney of the Swiss Albino Mice was stained and viewed under the microscope for toxicological study.

The phytochemical screening of stem bark of *Alstonia boonei* and *Capsicum frutescens* fruit shows the presence of Tannins, Flavonoids, Resins, Saponins, Terpenes, Alkaloids, Glycosides while Anthraquinones and acid compounds are absent in the both plants. Similar results were recorded by Adebayo et al. and Irene et al. [6,25]. These constituents have been found in other natural products which exhibited antimalarial activity reported that plants which contain many phytochemicals with biological activities like alkaloids and flavonoids could serve as sources of antimalarial drugs [26].

Studies have recorded that some of these phytochemical compounds are antioxidants. This property of the plants has been implicated in creation of an intracellular environment that is unfavorable to *plasmodial/salmonella typhi* growth [27]. This suggested that the antiplasmodial/anti-typhoidal properties of the synergistic extract could be based on the antioxidant, antiparasitic and antimicrobial effects of these phytochemicals [28]. This observation is validated by who reported that Artemisinin/Ciprofloxacin (a modern antimalarial/anti-typhoid drug) depends on its oxidant action for its potency against *Plasmodium* and *Salmonella* species [29].

These results obtained from Table 4 and 5 in agreement with shows that the plant extracts possesses antimalarial/anti-typhoid effect [30,31] also reported that ethanolic stem bark extract of *A. boonei* possesses potent antimalarial effect [32]. While the chemo-suppressive effect of aqueous extract was dose-insensitive, the ethanolic synergistic extract of *Alstonia boonei* and *Capsicum frutescens* exhibited a progressive dose-response relationship. The highest chemo-suppression (80%) observed at the highest dose (400 mg/kg/day) for aqueous extract is lower than the highest value (85%) observed at medium dose (200 mg/kg/day) for ethanolic extract [32]. This may be considered as an indication that the potency of synergistic ethanolic extract in the suppression of parasitaemia/typhoidal is more than that of aqueous extract. Relative to the value of 97% obtained for Chloroquine/Ciprofloxacin (an established anti-malaria/anti-typhoid drugs), ethanolic extract at 200 mg/kg/day effectively suppresses the infection.

The results obtained from this research work using the synergistic extract is consistent with the traditional use of parts work of the plants as ethnotherapeutic agent against malaria/typhoid in West and Eastern Nigeria as reported by Igoli et al. [33]. The synergistic

therapy was very effective and was also dose-dependent. The higher potency attained by the synergistic therapy at the maximum doses administered (200 mg kg⁻¹ and 400 mg kg⁻¹ b.wt.) may be due to the presence of certain phytochemical compounds which were present in the two extracts [34]. For instance, saponins, flavonoids and steroids were present in both extracts and their synergistic effects might have caused the potency against malaria/typhoid.

Toxicology and histopathological study of synergistic ethanolic extract of *Alstonia boonei* and *Capsicum frutescens* on the liver enzymes and kidney function herbal remedy which in most cases is a combination of two or more plants is usually taken for the treatment of malaria or typhoid and other ailments most especially in Africa. This good intention but with fatal consequence has its deleterious effect in terms of toxicities. The result obtained from Table 5 and 6 showed increased activities on the liver enzymes and kidney functions on administration of the synergistic extract.

The hematological parameters did not fall below a threshold which could pose danger to the animals, however, an increase in these hematological parameters following the withdrawal of the drug candidates showed that; administration of the extract had reducing effects on them. A basal level of AST and ALT is found in the plasma which may increase when there is damage caused on the liver and kidney. Increase in the serum level of ALP indicates liver injury or hepatitis [35]. The reference values for AST (0-40 U/L) and ALT (0-45 IU/L) in human [36] showed that all values obtained for these enzymes from all the drug candidates at both doses and at 7 days and 21 days were significantly higher than normal and it was evidently clear that damage was done to the liver. The increase in the activities of these enzymes after 7 days is indicative of the toxic effects of the extract of the stem bark extract of *A. boonei* subsequent decrease in the serum activities of these enzymes after 21 days did not reveal complete recovery [36].

Microscopic examination of the liver and kidney sections after the recovery period showed moderate atrophy of the hepatic cord and accentuation of sinusoids at a lower dose of 1000 mg/kg, Centrilobular hepatocellular generation, necrosis and inflammation 2000 mg/kg and 4000 mg/kg more so, there is tubular epithelial coagulation necrosis and attenuation on the kidney. The congestion observed in the liver is evidence that the liver is involved in the biotransformation of xenobiotic [36].

The kidney is an organ for excretion; therefore, it is exposed to both metabolized and un-metabolized toxicant for their removal from the body; the heavy task for the removal of toxicant from the body may cause much damage for the kidney. Again, some herbs may contain compounds such as oxalate which may chelate calcium and thus cause kidney stone. This means that high dose of synergistic ethanolic extract of stem bark extract of *Alstonia boonei* and *Capsicum annum* is both hepatotoxic and nephrotoxic and 21 days after the withdrawal of the drug did not give complete recovery. The treatment of the negative control groups with water did not show any noticeable effects on the hematological parameters, marker enzymes and histological evaluation [37,38].

However, the potency of the synergistic ethanolic extracts of *Alstonia boonei* and *Capsicum frutescens* at different dose levels were not in doubt as up to 91.7% suppression of malaria parasite and typhoid. These extracts could be utilized for the trial of some newer antimalarial/anti-typhoid drugs in future in view of the constant

development of resistance of malaria to currently used drugs. This same extract should be utilized with caution as it is dose sensitive and may cause hematological damage to the liver and kidney [39].

Conclusion and Recommendation

Taking together, the results obtained from this research work shows the efficacy of a synergistic ethanolic extract of *Alstonia boonei* and *Capsicum frutescens* compared with aqueous extract to cure malaria and typhoid in a Swiss Albino Mice. Ethanolic extract of *Alstonia boonei* and *Capsicum frutescens* is suggested to be very effective in the treatment of malaria/typhoid.

This research work suggested a moderate intake of herbal formulation for the treatment of malaria/typhoid because of the possible side effects. The synergistic extract of *Alstonia boonei* and *Capsicum frutescens* are not only toxic, but also recovery from the toxic effects is not within a short-term limit. Although, administration of herbal medicines may be useful in the treatment of diseases, the secondary adverse effects must not be overlooked because in some cases, these side effects are more deleterious than the diseases these phytochemicals are originally used to treat. In conclusion, the safety of the administration of herbs must be considered when using them for therapeutic and prophylactic purposes.

Recommendation

In view of this finding, efforts should be made to further:

- Characterize the active components of this plant; and
- Elucidate the mechanisms of action of its components on malaria parasite and typhoid.
- Experimentally show the lethal dose to be used.

Acknowledgement

All the technical staffs of the laboratory unit of Both the Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria, for their support and all the technical assistance rendered during the course of this research work.

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