*South Asian Research Journal of Natural Products*

*Volume 6, Issue 3, Page 157-168, 2023; Article no.SARJNP.100806*

# Evaluation of Anti-*Salmonella* Activity and Acute Toxicity of *Azadirachta indica* (A. Juss) Seed Oil

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors YMP, EAT and CF conceived and designed the study and drafted manuscript. Authors MGA, BH, GKH and NNB coordinated laboratory analysis and data assembly. Authors YMP and BH did the data mining. All authors read and approved the final manuscript.*

***Article Information***

**Open Peer Review History:**
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***Original Research Article***

***Received: 02/04/2023***
***Accepted: 07/06/2023***
***Published: 24/07/2023***

## ABSTRACT

Bacterial infections by *Salmonella* remain a real public health threat causing each year, more than 1.2 million deaths worldwide. The treatment by antibiotics is usually compromised due to the emergence of multi-resistant strains, supply chain problems and the high cost of medicines in developing countries where infectious diseases are usually endemic. Faced to these difficulties,

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these populations turn to medicinal plants treatment and essential oils, whose uncontrolled use poses effectiveness and safety issues. Using agar diffusion and macrodilution methods, we selected three *Salmonella* strains to evaluate the antibacterial activity of *Azadirachta indica* seed oil. Subsequently, an acute toxicity study of the extract was performed on Wistar rats by force feeding of the test groups with 2 ml/100kg of body weight, according to the slightly modified Organisation for Economic Co-operation and Development (OECD) guideline 420. Two of the three bacterial strains were susceptible with diameters of 17mm for *Salmonella typhi* and 18,5mm for *Salmonella paratyphi.* Their minimum inhibitory and bactericidal concentrations were 37.5mg/ml and 150mg/ml respectively with a MBC/MIC ratio equals to 4. Acute toxicity analyses showed that the lethal dose 50 (LD 50) of this oil was greater than 2ml/100g. *Azadirachta indica* seed oil possessed anti-*Salmonella* bacteriostatic properties on two strains and it was toxicologically safe at the dose of 2 ml/100g of body weight.

*Keywords: Anti-salmonella; acute toxicity; Azadirachta indica seed oil.*

## 1. INTRODUCTION

"Bacterial infections caused by *Salmonella* are responsible for more than 1.2 million deaths in the world annually. They continue to be a public health threat in developing countries in general and in Sub-Saharan Africa in particular, where they are endemic" [1,2]. About 11.9 to 26.9 million cases of typhoid fevers and 129 to 270,000 typhoid-related deaths have been estimated to occur worldwide each year [3]. In Cameroon, typhoid fever is endemic with limited episodes in time and space. Recent data shows that in December 2016, 262,149 cases were recorded nationally compared to 220,337 cases in 2015 [4]. *Salmonella* can cause typhoid and paratyphoid fevers, gastroenterics, individual and community food poisoning, lethargy and other extra-intestinal complications in humans [5]. In some developing countries, phenicoles, aminopenicillins and cotrimoxazole remain the reference treatment option for these infections. However, the emergence of multidrug-resistant *Salmonella* strains has led to new treatment regimens favouring parenteral cephalosporins, fluoroquinolones and sometimes azithromycin [6]. These multi-drug resistant serotypes pose a serious problem to the treatment of salmonellosis. Hence, there is a need to develop new active molecules for better preventive and curative management [7]. "With the rising phenomena of antimicrobial resistance, many medicinal plants were screened for their possible antimicrobial action" [8]. "Furthermore, plants contain a wide range of biologically active molecules, making them rich sources of various medications; consequently, it is necessary to identify and evaluate such plants with potential antimicrobial activities" [9].

"The emergence of drug resistant bacteria is a matter of concern as it is a serious global problem, as well as clinicians and the pharmaceutical industry. Although modern medicine has made great improvements in recent decades, about 80% of the population in developing countries still rely on medicinal plants, which occupy an important place in therapy" [10]. "The use of herbal medicines in the developing world continue to rise because they are rich source of novel drugs and their bioactive principles form the basis in medicine, pharmaceutical intermediates and important compounds in synthetic drugs" [11]. "Many researchers have documented the potent activity of plants' bioactive compounds on drug-sensitive and resistant bacteria" [12,13]. "*Azadirachta indica* A. Juss, a member of the *Meliaceae* family commonly known as Neem, is a tree mainly used in India to make all sort of consumer products, such as pesticides and insect repellents, soaps, cosmetics, toothpaste, antiseptic, gargle, ointment, poultries, lubricants, fertilizers, fuel for oil lamps, rope, glue and tannin from bark fibre etc" [14,15]. Various parts of this plant have been used for centuries in traditional medicine as a household remedy for the treatment of several diseases in many localities around the world, especially in India, South Asia and Africa [16,17]. In Cameroon, *Azadirachta indica* is mostly found in the Far North region where it is mainly used as a bio-pesticide in the protection of vegetable and seed crops [18]. It is a powerful disinfectant and in the Far North region of Cameroon as in other Sahelian regions in Africa, *Azadirachta indica* leaves are used to treat malaria, leprosy and gastroenteritis [19]. The fruit and bark are also part of the therapeutic arsenal. Rich in azadirachtin, the oil extracted from the seeds is used as a dewormed, antihypertensive, anti-hyperglycaemic and antibacterial; but given its use as a biopesticide [20], it exhibits some cytotoxic effects in humans [21]. Thus, the extensive use of Neem oil raises many questions about its safety, quality, efficacy and long-term availability. So far, there are few studies in Cameroon demonstrating its safety to the organism. Objectives of this study were to evaluate the anti-*Salmonella* effect of the extract of *Azadirachta indica* seed oil on three bacterial isolates, as well as determine its acute toxicity.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of the Plant Material

Oil from *Azadirachta indica* seeds was collected from the fruits of a mature tree in the Far North of Cameroon in the town of Yagoua. The fruits were taxonomically identified and authenticated by the National Herbarium of Cameroon as fruits of *Azadirachta indica* and the identification code assigned to the sample was 4447/SRFK. Traditionally, *Azadirachta indica* oil was obtained after crushing the kernels by cold pressing. The yield was calculated according to the following formula:

$$R=mb/mi$$

*mb: mass of crude oil*
*mi: initial seed mass*

### 2.2 Evaluation of the Antibacterial Activity

It was done by determining the inhibition diameters in solid medium by the disc diffusion method and by determining the minimum inhibitory and bactericidal concentrations in liquid medium by macrodilution according to the recommendations of the CA-SFM 2019.

### 2.3 Preparation of the Inoculum

Using a sterile platinum loop, we collected 2 to 3 colonies aged 18 to 24 hours and made a suspension with sterile physiological water (normal saline) similar to a 0.5 McFarland standard, corresponding to the concentration of 108 Colony Forming Units/ml (CFU/ml). Inoculation was done within 15 minutes after preparation of the inoculum.

### 2.4 Preparation of the Oil Solution from *Azadirachta indica* Seeds to be Tested

The extracted oil solution of concentration 100 mg/ml was prepared each time it was to be used. This was done by dissolving 1 gram of the extract (obtained by weighing 1g of *Azadirachta indica* seed oil) in 10 ml of dimethylsulphoxide (DMSO).

### 2.5 Susceptibility Testing: Agar Disc Diffusion Method

To test for the susceptibility of the bacteria, the principle of aromatogram was used. 20 µL of *Azadirachta indica* oil supplemented with 10% dimethyl sulphoxide (DMSO) was introduced into a tube and homogenised with a vortex. Sterile discs of 6 mm in diameter cut in a Whatman n◦. 2 paper were then added into the tube. Once the discs were impregnated with *Azadirachta indica* oil, they were removed and gently placed on the surface of a Mueller Hinton agar plate previously swabbed with the bacterial inoculum.

Simultaneously, Ciprofloxacin 500 mg antibiotic discs were also deposited on the same agar as a positive control. The plates were then incubated for 24 hours under strict anaerobic conditions. Following incubation, the discs were surrounded by circular zones of inhibition corresponding to an absence of culture, which allowed us to measure the diameters of inhibition using a calliper. This experiment was performed trice for each bacterial strain.

The determination of the diameter of the zone of inhibition enabled a clinical categorisation (S, I, R) of the strain, based on the correlation between the results obtained by the diffusion method and the reference method. The susceptibility to the oil is classified according to the diameter of the zones of inhibition as follows: Not susceptible (-) for diameter less than 8 mm; susceptible (+) for diameter between 8-13.9 mm; Very susceptible (+ +) for diameter between 14-19 mm; extremely susceptible (+++) for diameter over 19 mm. This susceptibility test allows access to qualitative results. The experiment was repeated three times.

### 2.6 Determination of Minimum Inhibitory Concentrations (MIC) and Bactericidal Concentrations (MBC)

The minimum inhibitory concentration (MIC) was expressed as the lowest dilution that inhibits growth due to the lack of turbidity in the tube. After recording the lowest MIC, all tubes not showing visible growth, as well as the control tube, were subcultured and incubated at 37°C for

18 hours. The minimum bactericidal concentration (MBC) is the lowest concentration at which a substance is capable of killing more than 99.9% of the initial bacterial inoculum after 24 hours of incubation at 37°C. Thus, the determination of the MBC is based on subculture from the MIC on agar. The solution from each of the tubes without bacterial pellets and form the positive control with the concentration range performed for the determination of MIC was inoculated by streaking on Mueller Hinton agar. The plates were then incubated for 24 hours at 37 °C. The MBC of the Neem oil is deduced from the lowest concentration at which no culture is observed on Mueller Hinton agar. This procedure was repeated trice for each bacterium.

### 2.7 MBC/MIC Ratios

The MBC/MIC ratio made it possible to determine the bacteriostatic or bactericidal character of our *Azadirachta indica* oil. An essential oil is said to be bacteriostatic when this ratio is greater than or equal to 4 and bactericidal when this ratio is less than 4.

### 2.8 Acute Toxicity Assessment According to OECD Guideline 420

We used the slightly modified OECD guideline 420 which recommends the administration of a single dose of extract and observation of the animals for 14 days.

### 2.9 Preparation of Animals

Twenty rats consisting of ten males and ten females with a mean weight of (106.4±15.05) g and (114.6±17.71) g respectively were used for this study. For each gender, these animals were divided into two groups of five; that is; the test group and the control group. All animals were weighed before being allocated to the different groups and then put too fast for 24 hours. After 24 hours of fasting, the test groups received a concentrated extract solution of 2 ml/100g via a stomach tube and the control groups were administered distilled water only. Again, the animals were deprived of food for four hours during which their behaviour was observed, and during the 14 days of the experiment their water and food consumption, weight variation and behaviour were monitored and recorded. At the end of the 14 days, the animals were weighed just before their sacrifice, which took place after anaesthesia with excess of ether. A blood sample was taken from the carotid artery to determine the biochemical parameters of toxicity. The organs were isolated and weighed immediately. The livers and kidneys were fixed in 10% formalin for histological sectioning.

### 2.10 Analysis of Biochemical Parameters

The determination of the effect of *Azadirachta indica* seed oil on biochemical parameters was done by evaluating the serum level of the following parameters: Creatinine, urea, Total proteins, Alanine Amino transferase, Aspartate Amino transferase, Total bilirubin, Alkaline phosphatase and Gamma GT through colorimetric assay using BIOLABO assay kits and a semi-automatic BIOBASE-Silver Plus Spectrophotometer.

### 2.11 Histological Analysis

The histological techniques used in this work were basic techniques and consisted of: fixation, macroscopy, dehydration, inclusion, sectioning, staining and mounting [22]. Microscopic analysis was performed with the aid of an AxiosKop 40 microscope connected to a computer where images were transferred, edited and analysed with MRGrab 1.0 and AxioVision 3.1 softwares, all supplied by ZEISS (Hallbermoos, Germany).

### 2.12 Statistical Analysis

The results were expressed in terms of mean +/- standard deviation. Comparison between groups was performed using the analysis of variance test (ANOVA) followed by Turkey's Kramer's post hoc test using the Graph Pad Instat version 5.0 software.

## 3. RESULTS

### 3.1 Antibacterial Activity of *Azadirachta indica* Seed Oil

The disc diffusion method used helped determine the susceptibility of *Salmonella* bacterial strains to *Azadirachta indica* oil.

### 3.2 Diameters of the Zones of Inhibition of *Azadirachta indica* Seed Oil

The three bacterial strains identified were tested for susceptibility to the control antibiotic and to *Azadirachta indica* oil. Only two of the three bacterial strains tested were susceptible to the *Azadirachta indica* oil (Fig. 1).

Diameters of 6 mm, 17 mm and 18.5 mm were obtained. All these bacterial strains were susceptible to ciprofloxacin (Table 1).

### 3.3 Minimum Inhibitory and Bactericidal Concentrations of *Azadirachta indica* Seed Oil

The minimum inhibitory and bactericidal concentrations as well as the MBC/MIC ratio of *Azadirachta indica* oil are shown in Table 2. The MIC for all three strains was 37.5 mg/ml and the MBC was 150 mg/ml. The MBC/MIC ratio gave a value of 4. It was deduced that *Azadirachta indica* oil had a bacteriostatic action on the tested bacterial strains.

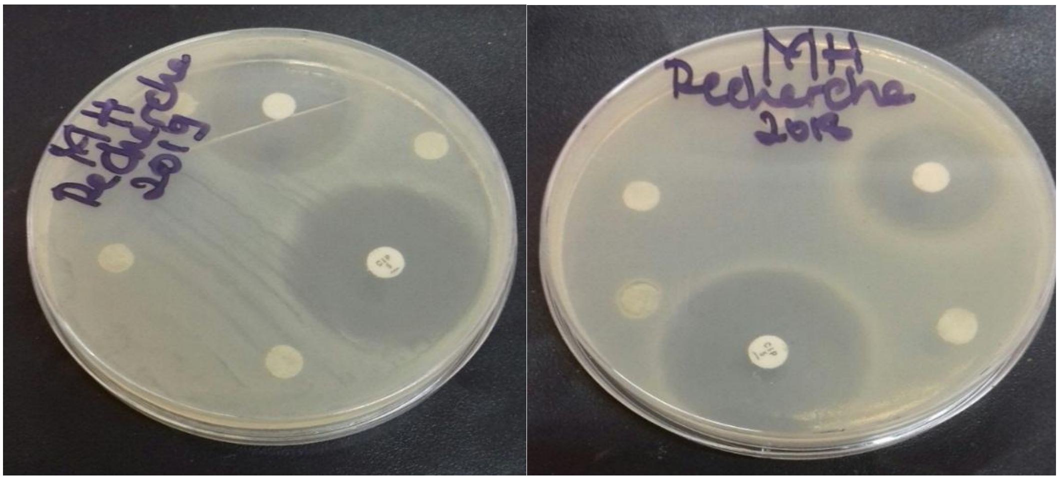
### 3.4 Acute Toxicity Assessment

**Extraction efficiency:** A yield of 8.567% was obtained from the extraction.

**Zootechnical criteria :** Analysis of Fig. 2 shows a weight gain among groups which received the extracted oil compared to those which did not with a p-value > 0.05. The results in Table 3 show a non-significant decrease in food intake. The same is true for water intake but with a significant decrease and a *p-value* < 0.05, in the female test group compared to the female control group.

**Comparative assessment of organs weight:** Analysis of the results in Table 4 did not reveal any significant difference in the relative weight of the organs between the different study groups.

**Assessment of biochemical parameters:** Analysis of transaminase results showed a non-significant increase in ALT levels in the male test group (90.70 ± 17.41) IU/L compared to the healthy control group (73.36 ± 18.61) IU/L. On the other hand, a non-significant decrease in activity was observed in the female test group (70.02 ± 7.17) IU/L compared to the female control group (88.32 ± 15.38) IU/L with a p-value > 0.05. The same results were observed for ASAT where we noticed a non-significant increase in enzyme activity in the male test group (206.80 ± 33.37) IU/L compared to the control group (201.00 ± 48.76) IU/L. In the female control group (278.75 ± 56.62) IU/L, we observed a decrease in enzyme activity (231.80 ± 49.25) IU/L with a *p-value* > 0.05. These results suggested the absence of hepatic cytolysis.



**A : *Salmonella typhi*,** **B : *Salmonella paratyphi A***

**Fig. 1. Antibacterial activity of *Azadirachta indica* seed oil (Photograph by Yede, 2019)**

**Table 1. Inhibition diameters of *Azadirachta indica* seed oil and ciprofloxacin on isolated bacteria**

| Bacteria | Ciprofloxacin 500 mg | *Azadirachta indica oil* |
|---|---|---|
| *Salmonella typhimurium* | 30.00 ± 2.52 | 6.00 ± 0,33 |
| *Salmonella typhi* | 32.00 ± 2.52 | 17.00 ± 0.26 |
| *Salmonella paratyphi A* | 34.00 ± 2.65 | 18.50 ± 0.51 |

**Table 2. MIC, MBC and MBC/MIC ratio values for strains tested**

| Bacteria | MIC (mg/ml) | MBC (mg/ml) | MBC/MIC |
|---|---|---|---|
| *Salmonella typhi* | 37.5 | 150 | 4 |
| *Salmonella paratyphi A* | 37.5 | 150 | 4 |
| Ciprofloxacin 500 mg | / | / | / |

*MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration.*

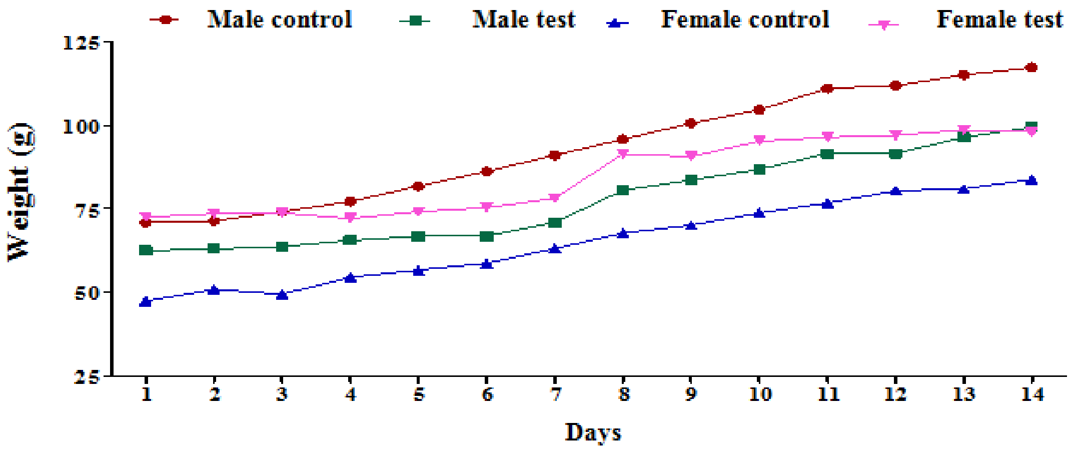



**Fig. 2. Kinetics of weight change in rats from different study groups**

**Table 3. Assessment of zootechnical parameters**

| Parameters | Male Control | Test male | Female Control | Test female |
|---|---|---|---|---|
| Food intake (g) | 88.31± 22.50 | 62.31 ± 32.83 | 76.31 ± 22.35 | 75.69 ± 32.54 |
| Water intake (ml) | 90.31 ± 18.54 | 75.69 ± 14.31 | 77.54 ± 18.73 | 56.31 ± 19.57 |
| Weight gain (g) | 46.20 ± 12.56 | 36.80 ± 10.47 | 36.20 ± 16.80 | 31.00 ± 16.79 |

**Table 4. Comparative assessment of the relative weight of organs**

| Organs | | Male control | Male test | Female Control | Female test |
|---|---|---|---|---|---|
| Heart | | 0.39 ± 0.05 | 0.42 ± 0.05 | 0.40 ± 0.06 | 0.45 ± 0.08 |
| Liver | | 3.67 ± 0.28 | 4.32 ± 0.58 | 4.07 ± 0.30 | 3.70 ± 0.32 |
| Lungs | | 0.92 ± 0.25 | 0.79 ± 0.17 | 0.78 ± 0.07 | 0.94 ± 0.24 |
| Brain | | 1.28 ± 0.22 | 1.56 ± 0.18 | 1.70 ± 0.50 | 1.58 ± 0.56 |
| Spleen | | 0.90 ± 0.22 | 0.90 ± 0.10 | 0.66 ± 0.29 | 0.72 ± 0.21 |
| Kidneys | Left | 0.36 ± 0.04 | 0.39 ± 0.03 | 0.43 ± 0.05 | 0.40 ± 0.03 |
| | right | 0.38 ± 0.04 | 0.41 ± 0.03 | 0.46 ± 0.03 | 0.45 ± 0.06 |
| Testis/Ovaries | Left | 0.61 ± 0.15 | 0.64 ± 0.08 | 0.06 ± 0.04 | 0.06 ± 0.03 |
| | Right | 0.62 ± 0.15 | 0.66 ± 0.10 | 0.06 ± 0.03 | 0.06 ± 0.02 |
| Adrenals | Left | 0.02 ± 0.01 | 0.02 ± 0.005 | 0.03 ± 0.01 | 0.02 ± 0.008 |
| | Droit | 0.02 ± 0.003 | 0.02 ± 0.004 | 0.03 ± 0.02 | 0.03 ± 0.02 |

Hepatic cholestasis parameters showed a non-significant decrease in Alkaline phosphatase activity in the male test group (569.80 ± 192.51) IU/L compared to the male control group (607.75 ± 32.25) IU/L with a *p-value > 0.05*. In females, we observed a significant decrease in the test group (485.60 ± 115.17) IU/L compared to the control group (777.20 ± 206.03) IU/L with a *p-value < 0.05*. Gamma GT analysis showed a significant elevation in the male test group (3.43 ± 2.01) g/l compared to the male control group (3.08 ± 1.22) g/l and the same result was observed in the females or in the female test group. A value of (4.59 ± 1.72) g/l was obtained compared to the control group (2.86 ± 1.46) g/l with a *p-value > 0.05*. Observation of Total bilirubin level gave a non-significant increase in activity in the male test group (7.16 ± 1.72) mg/L compared to the male control group (5.97 ± 1.34) mg/L. Compared to the female control group (8.10 ± 0.71) mg/L we recorded a non-significant decrease in activity (6.66 ± 2.23) mg/L in the female test group with a *p-value > 0.05*. These results suggested an absence of hepatic cholestasis following oil administration.

Analysis of renal damage parameters showed for Creatinine, a non-significant increase with a *p-value > 0.05* in the male test group (4.38 ± 0.58) mg/L compared to the male control group (4.32 ± 0.32) mg/L. However, a difference was noted in females with a non-significant decrease in the female test group (4.54 ± 0.15) mg/L compared to the female control group (4.84 ± 0.31) mg/L.

A non-significant increase in Urea level with a *p-value > 0.05* was observed in the male test group (0.64 ± 0.02) g/L compared to the male control group (0.49 ± 0.06) g/L. Also, similar results were recorded for the female test group (0.61 ± 0.08) g/L compared to the female control group (0.59 ± 0.10) g/L. The observed Creatinine and Urea activity suggested no renal involvement.

Moreover, a non-significant decrease in Protein level with a p-value > 0.05 was observed in the male test group (53.54 ± 5.56) g/L compared to the male control group (56.34 ± 2.59) g/L, and in the female test group (52.68 ± 3.73) g/L compared to the female control group (59.10 ± 3.34) g/L. This result suggested a possibility of oil-induced malnutrition.

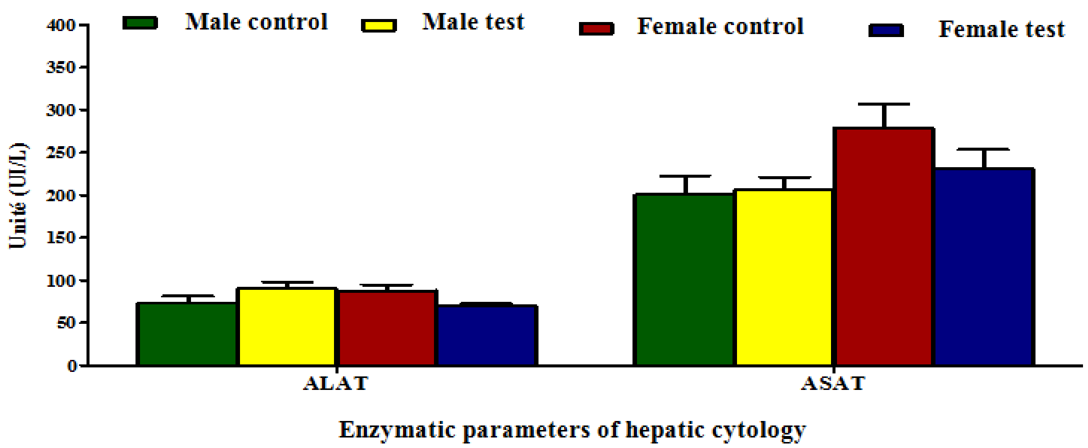



**Fig. 3. Effect of *Azadirachta indica* oil on serum transaminases**

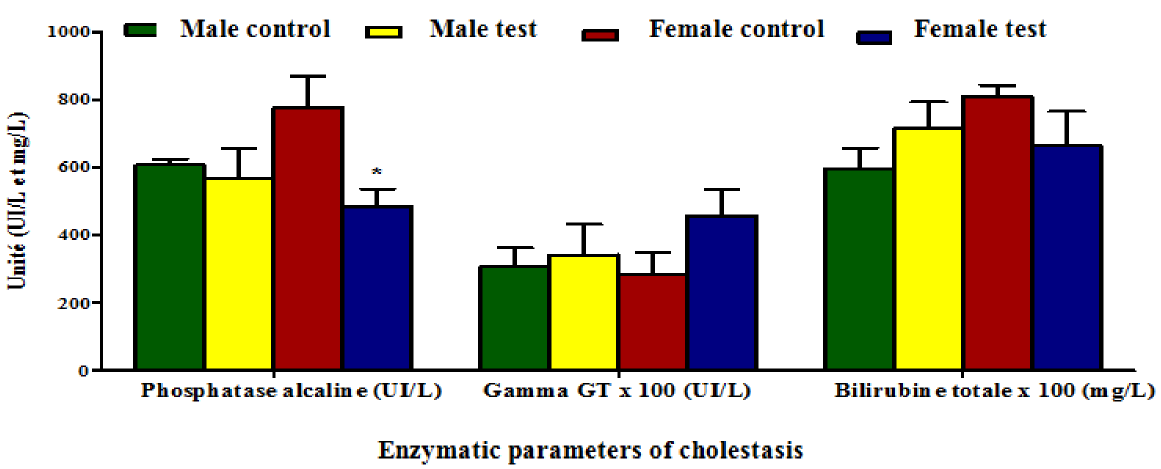



**Fig. 4. Effect of *Azadirachta indica* oil on liver cholestasis**

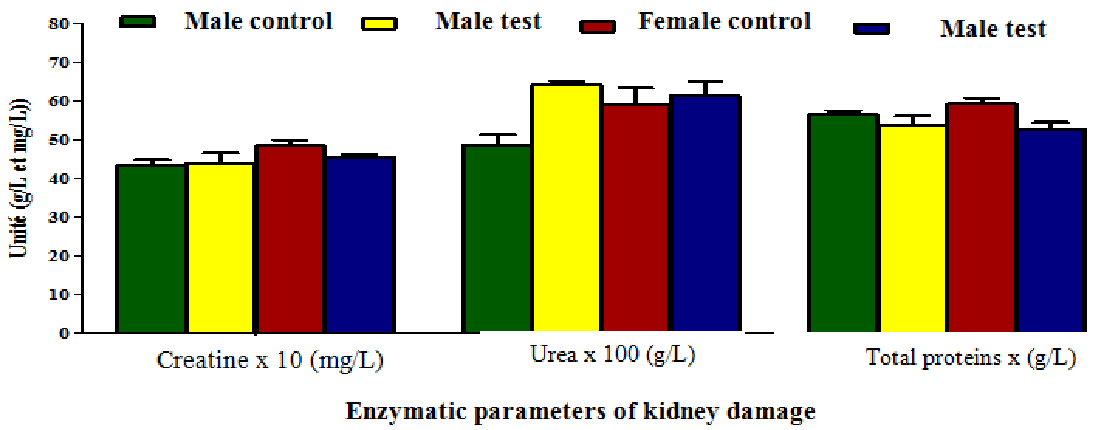



**Fig. 5. Enzymatic parameters of kidney damage**

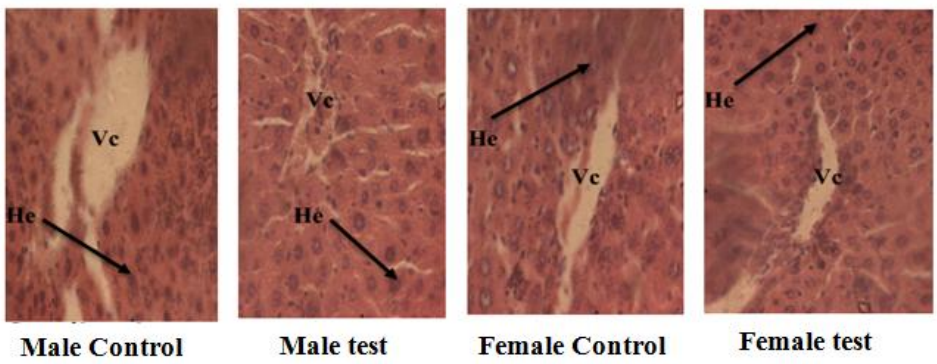



**Fig. 6. Liver histology for animals in the different study groups**
*Vc = Centrilobular vein (middle hepatic vein); He = Hepatocyte*

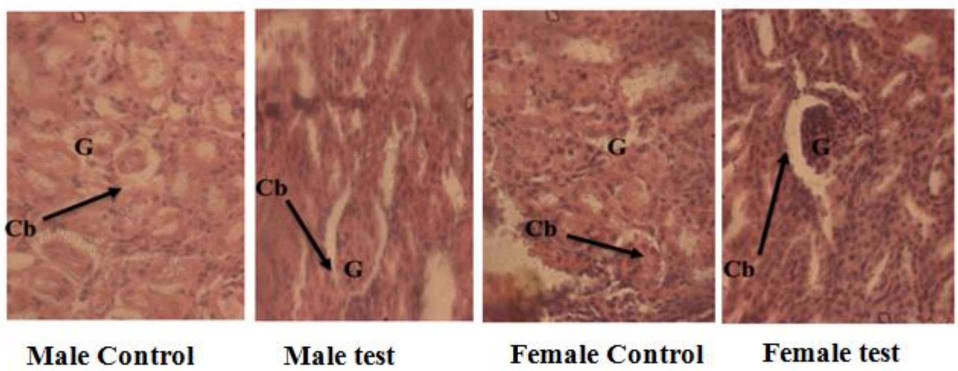



**Fig. 7. Kidney histology for animals in the different study groups**

**Assessment of histological parameters:** Results from histological sections showed no major changes in internal organ structures between the study groups. However, there was a slight obstruction of the middle hepatic vein in the male test group, which may suggest mild hepatic damage.

## 4. DISCUSSION

*Azadirachta indica*, the Neem tree, belongs to the mahogany family Meliaceae, it is native to Indian subcontinents and most of the African countries and is used in traditional medicine as a source of therapeutic agent. Neem ingredients are applied in Ayurveda, Unani, homeopathy and modern medicine for the treatment of many infectious, metabolic, or cancer diseases. Among all the parts of *Azadirachta indica*, the seeds are listed as one of the most popular source of medicaments in antibacterial activity as the oil contains extensive spectrum against bacterial infections [23]. To test the antimicrobial activities of Neem oil extracts *in vitro* methods such as broth dilution, disc or agar diffusion and agar overlay assays are commonly used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each treatment [24]. This study aimed to assessing the anti-*Salmonella* potential of the extracted oil from seeds of *Azadirachta indica* revealed inhibition of growth for some tested organisms. Two out of the three *Salmonella* strains were susceptible to *Azadirachta indica* seed oil with varying diameters. Thus, the determination of the inhibition diameter gave a result of 17 mm for *Salmonella typhi*, a same inhibition average (17.5 mm) obtained by certain studies [23] .This diameter is higher than those found in other studies, 8 mm [25] and 10 mm [26]. This difference could be explained by the fact that both oils were sourced from different regions and extraction methods were also different. However, the diameter obtained shows that *Salmonella typhi* is susceptible to our oil. *Salmonella paratyphi* A showed an inhibition diameter of 18.50 mm. We did not find any studies similar to ours reporting the inhibition diameters of *Salmonella paratyphi* A strain by *Azadirachta indica* oil. However, according to Moreira's classification, both bacterial strains are susceptible to *Azadirachta indica* seed oil. The values of the minimum inhibitory and bactericidal concentrations for the two bacterial strains studied were 37.5 mg/ml and 150mg/ml respectively. The ratio of the minimum bactericidal concentration to the minimum inhibitory concentration was 4. These values are different from those found by studies in China which obtained a ratio lower than ours [26]. This difference can be explained by the fact that they used the broth microdilution method. Therefore, our oil has a bacteriostatic antibacterial activity. This could be related to the presence of some compounds such as: lineoleoyl chloride 10.24%, methyl petroselinate 10.23%, phytol 9.6%, hentriacontane 8.8%,7-hexyl eicosane 8.1% and many others revealed by gas chromatography.

Given that Neem-based preparations have been consumed for several millennia, the normal consumption of Neem products can be regarded as absolutely safe [27]. It is assumed that the consumption of Neem products is absolutely safe since its preparations have been consumed over several generations but this assumption may have influenced the indiscriminate use of these products to a large extent amongst the rural population. These products are usually consumed over a long period of time without proper dosage monitoring by the experts and lack of awareness of the toxic effects that might result from such prolonged usage [27]. Scientific knowledge towards oral toxicity is much needed, which will not only help identify doses that could be used subsequently, but also to reveal the possible clinical signs elicited by agents under investigation. Hence, the current study was also undertaken also to evaluate the acute toxicity of *Azadirachta indica* seed oil in an animal model. During such evaluation, the determination of LD50 is usually an initial step to be conducted. The acute toxicity study may provide initial information on the mode of toxic action agent, acts as the basis for classification and labelling, and helps in deciding the dose of novel compounds in animal studies [26,28]. At a single dose of 2 ml/100g, *Azadirachta indica* seed oil had no adverse effects on treated rats during 14 days of observation. A non-significant decrease in food intake was observed in the test groups and in water intake in the male test group. However, a significant decrease in water intake was observed in the female test group. There was a non-significant weight gain in both test groups but the relative weight of the internal organs of the rats showed no difference between the test and control groups. These results correlate with other studies carried out in Cameroon, Nigeria and China which also reported this decrease during the second week. These studies suggest that the changes in water intake and in weight of the rats during the observation period may have occurred because the *Azadirachta indica* seed oil interfered with the absorption of nutrients making them unavailable or the oil intake may have made them feel satisfied for a while thus reducing their food intake [29-31]. The biochemical results of ALT, ASAT, Alkaline phosphatase, Total bilirubin, Creatinine, Urea and Total protein were not significantly altered between animals in the test and control groups after 14 days of extract administration. Other studies found in the literature presented similar results to ours [31]. Significant changes were found in Gamma GT levels but these do not necessarily indicate cholestasis or liver damage. However, a study conducted in Cameroon by Ngum *et al*. found a significantly elevated serum transaminase level and a statistically significantly decreased in total protein level [25]. This result could be explained by the difference in the dose administered in each study. Ngum *et al* administered a dose of 3 ml/100g [25].

The histopathological examinations of liver and kidney principally and of the internal organs of animals belonging to all groups showed normal integrity of these tissues and no apparent damage or lesions. However, the increase of transaminases in the present study was also accompanied by histological abnormalities such as slight obstruction of the central vein of the liver observed in the male test group which could suggest a slight hepatic injury. These results are consistent with those reported by other authors [32,33]. Therefore, the *Azadirachta indica* seed oil used does not cause acute toxicity effects at the dose tested and with LD50 values below 2 ml/100g. Nevertheless, these findings do not correlate with earlier studies where the LD50 of *Azadirachta indica* seed oil in rats was 3 ml/100g [25,33]. *Azadirachta indica* seed oil is reported to be hepatoprotective, and its hepatoprotective effect is dose-dependent. Some studies revealed that *Azadirachta indica* seed oil at 0.25 ml/kg, 0.5 ml/kg and 1.0 ml/kg was hepatoprotective, but could become toxic at a comparatively higher dose [34].

Oil extracts and their phytochemicals from the *Azadirachta indica* seed have significant anti-*Salmonella* activity against a multitude of pathogens that affect human health. In order to develop realistic *Azadirachta indica*-based treatment regimens that could be used in humans, there are clearly many intriguing areas for future investigations. Undoubtably, future experiments will need to elucidate the mechanisms of action of Neem, the associated phytochemicals and its toxicity. Some level of standardization should be considered so that comparisons can be made and patterns can be recognized across multiple studies. This may become easier when the antimicrobial activities of more individual phytochemicals are determined [35]. *Azadirachta indica* represents a novel source of antimicrobials that may be used to combat drug resistance and emerging threats to human health. Furthermore, the research that has been done on the Neem tree can be used as a guide to encourage the investigation of other traditionally used natural products for their utility as modern pharmaceuticals. Of note in recent years, there are several groups who have incorporated *Azadirachta indica* into novel materials and technologies that have broad implications for human health. Specifically, green-synthesized copper or silver nanoparticles and hydrogels, nanocellulose films, chitosan-copper oxide biopolymers, and hydroxyapatite have all been constructed to include *Azadirachta indica* extracts and have substantial antimicrobial activity, including against multidrug-resistant bacterial species [35,36].

## 5. CONCLUSION

This study whose aim was an evaluation of the anti-*Salmonella* activity and acute toxicity of *Azadirachta indica (*A. Juss) seed oil helped determine the bacteriostatic activity of our oil and that showed that the two strains were susceptible. Acute toxicity study concluded that the LD50 of this extract was greater than 2 ml/100g as no animal deaths were recorded during the experiment. Also, according to the results of the analyses obtained, taken at low doses over a short period of time, this oil would have no notable toxic effects on the liver and kidneys. The problem associated with salmonellosis contributed by pathogenic bacteria had increased resistance with commercial drugs and therefore could be solved by developing the *Azadirachta indica* seed oil as one of the active drug or a promising good alternative to fight against certain salmonellosis. This oil had been used for many other purposes in the traditional medicines and had been proven to be effective as anti-*Salmonella* agents.

## ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

## ACKNOWLEDGEMENTS

The Bacteriology Laboratory of the University Hospital Centre (CHU) of Yaounde I. The Laboratory of Animal Preclinical and Toxicological Studies of the Faculty of Science of the University of Yaounde I.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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