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ANTI-INFECTIOUS AND *IN VIVO* ANTIOXIDANT ACTIVITIES OF *ALBIZIA GUMMIFERA* AQUEOUS STEM BARK EXTRACT AGAINST *SALMONELLA TYPHI*-INDUCED TYPHOID FEVER IN RATS

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ABSTRACT

Typhoid fever is one such disease, still remains a serious problem and a major threat to the society in most of the developing countries. In our search for therapeutic agents from natural sources with potential for the treatment of typhoid fevers, this work aims to evaluate the *in vivo* antisalmonellal activity and the effect of bacterial infection on tissues oxidation of aqueous extract of *Albizia gummifera*. The *Salmonella typhi*-infected rats concurrently received different doses of plant extract (20, 40 and 80 mg/kg bw) or oxytetracyclin (5 mg/kg) daily for 9 days. The parameters such as, catalase peroxidase, nitric oxide, lipid peroxidation index, malondialdehyde, alkaline phosphatase and bilirubin were assessed. Results obtained showed that groups of treated animals at 20, 40 and 80 mg/kg bw of extract were healed respectively after 8, 7 and 6 days of treatment for females and after 9, 7 and 7 days of treatment for males. Antioxidant activity has shown that CAT (male) and peroxidase (both sex) activities for treated animals were increased significantly ($p < 0.05$) compared to the untreated control group animals while NO, MDA, ALP and bilirubin levels were significantly decreased ($p > 0.05$). Increase and reduction of theses antioxidants parameters to their normal value (that of the neutral control) was doses-dependent manner. The overall results of this study indicate that aqueous extract of *Albizia gummifera* exhibited in different in vivo systems, strong antisalmonellal activity and antioxidant activity on cell oxidation induced by infection with *Salmonella typhi.*

Keywords: *Albizia gummifera*, *Salmonella typhi*, bacterial infection, tissue oxidation, antioxidant activity

INTRODUCTION

Typhoid fever also known simply as typhoid, is a bacterial infection due to *Salmonella typhi* or *Salmonella enterica* serotype *typhi* that causes symptoms that may vary from mild to severe and usually begin six to thirty days after exposure.[1,2] Conventional antityphoid drugs are becoming more and more unavailable[3] because typhoid still remains a serious problem and a major threat to the society in most of the developing countries in general and in sub-Saharan Africa in particular, where it is endemic.[4,2] This problem is due to their emergence of multidrug resistance.[5] The occurrence of resistant microorganisms has consequently increased frequency of treatment failures and increased severity of infections.[6] Nearly 22 million cases of typhoid fever caused by *S. typhi* or *S. paratyphi* and 216 500 deaths is estimated annually.[7] Treatment of this disease is with antibiotics such as azithromycin, fluoroquinolones or third generation cephalosporins. Resistance to these antibiotics has been developing, which has made treatment of the disease more difficult.[6] Furthermore, many microbial infections such as typhoid fever lead to the production of highly reactive molecules from the metabolism of oxygen.[8] The increased formation of these highly reactive molecules that are deleterious to membrane lipids

lead to the cells oxidative stress.[8,9] Thus there is a need to search for new substances with interesting antityphoid and antioxydant activities.

Several plants used as a major source of health care by people in various traditional systems show immense medicinal potentials.[10,11,12,13] Among them, *Albizia gummifera* is traditionally believed to possess medicinal properties for various ailments such as bacterial infections, skin diseases, malaria and stomach pains.[14,15] It has been reported to exhibit anti-trypanosomal, anti-plasmodial, antibacterial and anticancer activities relative to the presence of spermine alkaloids, oleanane saponins and triterpenes.[16,17,18] In our search for therapeutic agents from natural sources with potential for the treatment of typhoid fevers, the present study was performed to establish the *in vivo* antisalmonellal activity and the effect of bacterial infection on tissues oxidation of aqueous extract stem bark of *Albizia gummifera* .

MATERIALS AND METHODS

Collection and Identification of Plant material: The stem bark of *A. gummifera* was collected in Foto, Menoua Division, West Region of Cameroon, in March 2013. The identification of the plant was done at the National Herbarium, in Yaounde-Cameroon, using a voucher specimen registered under the reference HNC N°20859/SRF-Cam by Ngansop Eric.

Microorganisms and culture media: In this study, one strain (*Salmonella typhi* ATCC6539) from the American Type Culture Collection (ATCC) was used. The culture media namely Salmonella-Shigella Agar (Italy Liofilchem) was used in this study for activation and maintenance of the strain.

Preparation of the plant: The leaves of *A. gummifera* were dried at room temperature (23 ± 2°C) until constant weight and powdered to coarse particles. One hundred grams (100 g) of powder were soaked in 1 L of distilled water and then boiled at 100°C for 15 min. At the end of the extraction, it was filtered through nylon mesh followed by Whatman No.1 filter paper. The filtrate was concentrated in a drying oven at 45°C to obtain aqueous decoction.

Animal treatment: Forty eight albino rats of both sexes, aged between 8 to 9 weeks were used. They were grouped randomly into six groups of six animals each (4 males and 4 females) with similar average body weight on the third day after acclimatization. Apart from animals of group 1, those of all other groups (2-6) were infected. They received orally 1 mL of a suspension containing $1.5x10^8$ CFU of *S. typhi*. Group 1 (NINT) served as a neutral control. Animals of group 2 (INT) were not subsequently treated and thus served as negative control. Group 3 (OXY) animals received on the fourth day after infection, oxytetracycline at 5 mg/kg (positive control) every day from the ninth day post-infection. Animals of other groups 4 to 6 were treated from the ninth day after infection with different doses of the plant extract (20, 40 and 80 mg/kg body weight). They were handled according to standard protocols for the use of laboratory animals. The studies were conducted according to the ethical guidelines of the Committee for Control and Supervision of Experiments on Animals (Registration no. 173/CPCSEA, dated 28 January, 2000), Government of India, on the use of animals for scientific research.

Sample collection: Rats were fasted overnight on the 9th day of treatment and urine was collected from individual metabolic cages, centrifuged and store at +4°C for 24 hours. Upon fasting, the blood samples were collected by cardiac puncture into non-heparinized tubes from chloroform anaesthetized rats. Animals were further sacrificed and used for gross pathological examinations and relative organ indices determination.

Biochemical analysis: Sera and organs homogenates were used for the determination of biochemical parameters related to oxidative stress such as catalase, peroxidase, malondialdehyde, nitric oxide; and serum alkaline phosphatase and bilirubin.

Endogenous antioxidant enzymes analysis: Two antioxidant enzymes were evaluated; Catalase (CAT) and Peroxidase (Px).

- **Catalase assay:** Catalase level was evaluated in serum and tissue by Dimo et *al*.[19] method. Ten microliters of the serum or tissues homogenate were added into 150 µl of phosphate buffer pH 7.4. Then 40 µl of H_2O_2 (50 mM) were also introduced. After 1 minute, 400 µl of potassium dichromate (5%) prepared in the 1% of acetic acid was introduced in the reactional solution. The mixture was heated in boiling water for 10 min and cooled immediately. The absorbance was recorded at 570 nm using spectrophotometer "Jenway, model 1605". Enzymatic activity of catalase was inferred by the Beer-Lambert law [20] in mmole/min per milliliter of serum or gram of tissue.
- **Peroxidase assay:** Peroxidase level was determined in tissue by Habbu et *al.*[21] method with slight modifications. Two hundred and fifty microliters of serum or organs homogenates was taken, and to this were added 500 µl of 10 mM KI

solution and 500 µl of 40 mM sodium acetate. The absorbance of potassium per iodide was read at 353 nm, which indicates the amount of peroxidase. Then 10 µl of 15 mM H_2O_2 was added, and the change in the absorbance in 5 min was recorded. Enzymatic activity of peroxidase activity was expressed in µmole/min per millliter of serum or gram of tissue by the Beer-Lambert law.[20]

Endogenous antioxidant markers analysis: Four antioxidant markers were evaluated: nitric oxide, malondialdehyde, bilirubin and alkaline phosphatase (ALP)

- **Nitric oxide (NO) assay:** The production of NO was estimated from the accumulation of nitrite (NO_2^-) using the Griess reagent, as previously described.[22] Briefly, equal volumes (170 µL) of samples or Nitrite Standard (0.1 M sodium nitrite in water) for standard curve and Sulfanilamide solution (1% sulfanilamide in 5% phosphoric acid) were mixed and incubated in obscurity at room temperature for 5 min. After the incubation, 170 µL of NED Solution (0.1% N-1 napthylethylene diamine dihydrochloride in water) is added and again incubated in the same condition. Nitrite, sulphanilamide and alpha - naphthylamine were purchased from Sigma. The absorbance values were measured at 520 nm using a spectrophotometer and the results were expressed in terms of micromoles of NO per gram of tissue or per milliliter of blood based on the standard equation of NO ($y = 0.0602x$; $r^2 = 0.9837$).

- **Measurement of lipid peroxidation index malondialdehyde:** The lipid peroxidation index was measured in tissue using Thiobarbituric Acid (TBA) test on Malondialdehyde (MDA) according to the method described by Oyedemi et *al.*[23] with some modifications. A total of 250 µL of 1% orthophosphoric acid and 250 µL of precipitating mixture (1% thiobarbituric acid, 1% acetic acid) were added to 50 µL of homogenate. The mixture was homogenated and heated in boiling water for 15 min and cooled immediately. It was then centrifuged at 3500×g for 15 min and the absorbance of the supernatant was recorded at 532 nm using spectrophotometer "Jenway, model 1605". The lipid peroxidation was calculated based on the molar extinction coefficient of Malondialdehyde (MDA) (153 $mM^{-1}cm^{-1}$), and expressed in terms of micromoles of MDA per gram of tissue or per milliliter of blood.

- Serum markers namely **alkaline phosphatase** and **bilirubin** levels were estimated based on colorimetric method using commercial kits (TECO DIAGNOSTIC Lakeview ave and IMNESCO Wiedtalstr, Germany respectively) and spectrophotometer "Jenway, model 1605".

Statistical analysis: Results were expressed as mean value ± standard deviation (S.E.M.). Within group, comparisons were performed by the analysis of variance using ANOVA test. Significant difference between control and experimental groups were assessed by Waller Duncan-test.

RESULTS

***In vivo* therapeutic test:** The aqueous crude extract of the stem bark was tested *in vivo* on a Salmonella *typhi*-induced typhoid in Wistar rats. The infected animals were observed to have loose and erect hairs (a sign of fever) and soft and mucous feacal matter (a sign of diarrhoea). Sometimes, the presence of blood and mucus made the stool to appear dark and shiny. These animals were weak and less active, with their fur standing at right angles to the body surface instead of the normal sleeping position. The slender body became more bulky, with some of the animals even 'coughing'. All of these characterized the establishment of infection in the experimental animals, which was clearly revealed by the growth of Salmonella colonies on Petri dishes after the culturing of blood.

Treatment with plant extracts improved the general condition of animals. The evolution of the bacterial load in the blood of experimental rats throughout the experiment is summarized in the Figure below. From the second to the sixth day after infection, the bacterial load continuously increased in the blood of infected animals. The healing effect of *A. gummifera* extract and reference antibiotic (oxytetracycline) was observed from the eighth day (the third day of treatment) since there was not a significant ($p > 0.05$) and dose-dependent decrease of bacterial load in both sex infected animals under treatment. Similarly, there was a slight decrease in bacterial load in negative control group animals but the load remained relatively and significantly ($p < 0.05$) high on the last day of treatment (10^5 for male and 1.4×10^5 for female) as compared to that of animals receiving different doses of extract. Animal groups receiving oxytetracycline (5), D1 (20), D2 (40) and D3 (80 mg/kg bw of extract) were healed respectively after 7, 8, 7 and 6 days of treatment for the females and after 7, 9, 7 and 7 days of treatment for the males.

Effect of Treatment on serum and organs catalase and peroxidase activities: The evolution of the activity of the lung, liver, spleen, heart, kidney and serum catalase (CAT) and peroxidase (Px) with different treatments is presented respectively in Table 1 and Table 2 below. It can be observed from this Table 1 that the infection resulted in a significant ($p < 0.05$) decrease in the activity of different organs and serum CAT in males, liver and heart in females; whereas other organs and serum CAT activity in females have not significantly ($p > 0.05$) changed as compared to neutral control. A relative and significant increase in both sex animals of CAT activity receiving different doses of *A. gummifera* extract compared to the negative control group animals was noted.

The peroxidase activity is presented in Table 2. From this table it can be noted that there is no significant ($p > 0.05$) difference in peroxidase activity of serum in males on one and liver, lungs and kidneys in females. Furthermore a relative and slight increase in peroxidase activity of the animals receiving different doses and neutral control animals was observed compared to the negative control animals. However the significant activity of peroxidase ($p < 0.05$) was noted between other organs and serum irrespective of sex in animals receiving different doses of extracts compared to the negative control. No significant activity of peroxidase ($p > 0.05$) was observed between the treated and neutral control animals.

Effect of Treatment on serum and organs NO and MDA activities: As illustrated by table 3 below, infection resulted in an increase in the rate of organs and serum NO compared to neutral control. This increase persisted in the untreated both sex animals group and decrease with the administration of different doses of *A. gummifera* extract in treated animals. Significant decrease level of NO ($p < 0.05$) was noted in serum and spleen of males compared to negative and neutral control. It was also observed in male treated animals receiving dose of 40 and 80 mg/kg a significant decrease in liver and lung NO levels ($p < 0.05$) respectively when compared to negative control. Significant increased levels of MDA ($p < 0.05$) was observed in the serum, heart and kidneys in male negative control, and liver, lungs, spleen and kidneys in female negative control compared to the neutral control and the animals receiving different doses (Table 4). However, the relative decrease of MDA levels in a doses-dependent manner was noted in the animals receiving different doses compared to the negative control group animals.

Effect of Treatment on serum bilirubin and alkaline phosphatase: The effect of *A. gummifera* on serum marker enzymes such as alkaline phosphatase, total and direct bilirubin, is presented in Table 5. The level of serum ALP significantly decreased ($p > 0.05$) in treated animals compared to the negative control. However, at doses 80 mg/kg for extract and 5 mg/kg for Oxytetracycline in males, it was significantly increased ($p > 0.05$) compared to neutral control but lower than negative control. The doses 40 mg/kg of ALP reduced to their normal value (that of the neutral control). It appears that infection resulted in an increase of total and direct bilirubin as compared to neutral control (table 5). Administration of different doses of *A. gummifera* and that of the mixture lead to a significant decrease ($p < 0.05$) in total and direct bilirubin compared to the negative control while treatment with *A. gummifera* extract at doses of 20 mg/kg and 40 mg/kg, showed a total and direct hyper-bilirubinaemia compared to the controls. However, at doses 80 mg/kg, total and direct bilirubin reduced to their normal value (that of the neutral control).

DISCUSSION

Typhoid is characterized by a high fever, colic pain, inflammation, hepatic injury and diarrhoea. *Salmonella typhi* has also been reported to cause hepatic dysfunction and hepatic abscess.[24] The establishment of infection was reflected by some changes in animals physiology including the excretion of watery stool, the presence of mucus in the stool, the reduction of its activity and the exponential increase in the rate of *S. typhi* in the blood of rats after administration of infectious load. This suggested that bacteria proliferated in the organs after having invaded the system, and challenged the non-specific defence mechanism of rats.[25] *Salmonella typhi* is an example of bacterium which enter the spleen, liver and other organs where it thrives and re-enter the blood when it escapes the macrophage cells.[8] The decrease of the bacterial load observed with treatment may be due to the combined action of the extract and immune system[25] and given the fact that this decrease was also noted in the negative control group (infected and untreated). Animals treated at therapeutic dose (20 mg/kg) recovered in almost the same period as those treated with multiples of that dose. This result suggests that this sample may have a higher *in vivo* activity due to their metabolism. *Albizia gummifera* stem bark phytochemical composition revealed the presence of several classes of compounds including alkaloids, saponins, phenols, flavonoids and anthocyanins. These secondary metabolites (flavonoids, alkaloids)

have already shown several pharmacological properties including antibacterial and antioxidant properties.[26,27] Bacterial infections in living cells release of toxins whose metabolism results may lead to an increased formation of highly reactive molecules that can cause oxidative damage thereby rendering the cells to oxidative stress.[8,9]

These tissues/organs are prone to damage by bacterial toxins which are released by bacterial cells to the host organism during the process of metabolism. This tends to disrupt the blood components/cells or blood forming tissues.[28] Equilibrium between ROS and enzymatic antioxidant enzymes such as catalase (CAT) and peroxidase (Px) for example are crucial and could be an important mechanism for preventing damage by oxidative stress.[29] Both enzymes play a significant role in the cellular defence against deleterious effects of free radicals. Catalytic removal of reactive oxygen species by CAT and Px enzymes are extremely important as they prevent numerous lipid peroxidation by-products, protein adduction and organ dysfunction.[29] A decrease in the activities of these enzymes could increase the availability of $O_2^{\cdot}$ and H_2O_2, which can lead to the production of $OH^{\bullet}$, subsequently initiating oxidative damage of the lipid membrane, proteins and DNA. Concurrently, increased CAT and Px allowed the effective removal of excess H_2O_2[30] to form water and molecular oxygen. NO is generated in biological tissues by specific nitric oxide synthases, which metabolizes arginine to citrulline with the formation of NO via a five electron oxidative reaction.[31] Peroxynitrite, results of the combination of NO with superoxide, also initiates oxidative damage like radicals hydroxyl OH^- by oxidizing lipids, nucleic acids, and proteins, distorting their structure and altering their function.

Catalase (CAT) is one of the important metallo-proteins in the supportive team of defence against reactive oxygen species (ROS).[32] Widely distributed in all animal tissues, it is a protective enzyme tissue from reactive hydroxyl radicals which accomplished their antioxidant functions by enzymatically detoxifying the peroxide (OH, H_2O_2) and superoxide anion.[29,32] This haemoprotein containing four haem groups, that catalyses the decomposition of H_2O_2 to water and O_2 and thus, protects the cell from oxidative damage by H_2O_2 and OH.[33] The inhibition of CAT activity may be due to the enhancement of the peroxidation end product TBARS, which is known to inhibit protein synthesis and the activities of certain enzymes.[33] Administration of *A. gummifera* aqueous extract and oxytetracycline enhanced the activity of CAT in salmonella – induced organs damage. The enhancement in CAT activity may be done to prevent the accumulation of excessive free radicals and protect organs from salmonella – intoxication as shown by Yakubu et *al*.[33] The significant increase CAT activity and decrease MDA level in the serum and organ of extract treated group, is an indication of effectiveness of the plant antioxidant. According to Coolborn et *al*.[32], this manifestation is evident because catalase converts harmful hydrogen peroxide into water and oxygen which in that order protects the organs tissue from highly reactive hydroxyl radicals able to be generated by *S. typhi* infection at a concentration of 10^7UFC/ml for the three days induction. The reduction in the activities of this enzyme as observed in the negative control rat may results in a number of deleterious effects due to accumulation of highly toxic metabolites and hydrogen peroxide by *S. typhi* in infection. This is liable to induce oxidative stress in the cells.[34] According to Sharida et *al*.[29], significant reduction of CAT activity indicates oxidative stress. The administration of the extract in various concentrations increased the activities of catalase in rat preventing the accumulation of excess free radicals thereby protecting the liver and other organs from intoxication induced by *S. typhi* infection.[32] Significant increase in CAT levels suggested that either the plant itself reacts with the reactive oxygen species or boosting the antioxidant enzyme production.[29]

Peroxidase is an enzyme that catalyses the reduction of hydroperoxides, including hydrogen peroxides, and functions to protect the cell from peroxidative damage.[36] Concomitant increase in peroxidase with CAT levels were found in salmonella infected animals while their levels were restored in the extract and oxytetracycline treated groups compared to neutral control indicating that extract can reduce reactive oxygen free radicals and improve the activities of the antioxidant enzymes.[36]

Malondialdehyde (MDA) is a good indicator of the degree of lipid peroxidation related to salmonella-induced tissue damage.[29] It is one of the end-products in the oxidative breakdown of polyunsaturated fatty acids, and is a marker used frequently to evaluate lipid peroxidation in tissues.[30] In the present study, the salmonella infection induced lipid peroxidation, suggesting increased oxidative stress. This could be due to increased free radical formation accompanied by reduced antioxidant enzyme and antioxidant activities.[30] Compared to negative control, concentration of MDA was relatively/significantly decreased by treatments with extract of *A. gummifera* in serum or all organs, indicating its ability to break the chain reaction of

lipid peroxidation in *Salmonella typhi*-exposed rats. Based on these results, we may suggest that the therapeutic potential of *A. gummifera* extract is dependent on an antioxidant mechanism. Membrane destabilisation by enzyme (lipoxygenase for example), which is known to oxidize polyunsaturated fatty acids and to produce free radicals, is generally attributed to lipid peroxidation, due to an increased production of toxic oxygen free radicals.[37] Our results indicate that *S. typhi* induces oxidative stress, as was evident the increased accumulation of lipid peroxidation products, in blood and all organs of stressed animals.

Nitric oxide (NO) is generated in biological tissues by specific nitric oxide synthases, which metabolizes arginine to citrulline with the formation of NO via a five electron oxidative reaction.[31] It is produced from L-arginine by the neuronal, endothelial, and inducible isoforms of the NO synthase (nNOS, iNOS, and eNOS). It is believed to participate in the regulation of the oxidation/reduction potential of various cells and may be involved in either the protection against or the production of oxidative stress within various tissues depending on its concentration. Compared to the negative control, significant decrease noted in treated animals receiving extracts or oxytetracycline leading to normalized level of NO shown that this plant extract prevents alteration of some biomolecules and of chronic degenerative diseases. According to its multiple physiological properties, NO at high concentrations exerts toxicity through its combination with superoxide anion to form peroxynitrite which induces nitration of lipids, nucleic acids, and proteins, distorting their structure and altering their function. Peroxynitrite - mediated cytotoxicity has a key role in the pathogenesis of a number of chronic degenerative diseases of the brain, heart, and liver.[38] Biliverdin reductase (BVR) mediates reduction of biliverdin (BV) to bilirubin (BR) which is the much more potent antioxidant. The subsequent oxidation of BR by hydrogen peroxide back to BV forms a catalytic antioxidant cycle driven by NADPH, the reducing cofactor of BVR.[39] As shown by Jansen et *al*.[40], bilirubin represents a superior antioxidant as compared to biliverdin when applied in high concentrations. Increased bilirubin in infected and untreated animals is a body response to fight against free radicals whose formation was induced by the infection. Indeed several studies have shown that free and bound bilirubin are potent radical scavengers and also protect human cells from lipid peroxidation.[41] These results corroborate those of Fodouop et *al*.[25], which suggest that bilirubin serves as a physiological antioxidant in *Salmonella typhimurium* infection on rat's cell oxidation.

Serum Alkaline phosphostase and bilirubin levels are related to the function of hepatic cell. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver.[42] Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure.[43] The treatment with *A. gummifera* extract in both sexes at the different doses significantly attenuated the elevated levels of the serum markers. This result suggests that *A. gummifera* extract is able to control of bilirubin level and alkaline phosphatase activity points in order to improve the secretory mechanism of the hepatic cells.[43]

The plant metabolite profiles of *A. gummifera* stem bark extract, possesses phenolic compounds and flavonoids, which are known to effectively scavenge free radicals. Antioxidants appear to act against disease processes by increasing the levels of endogenous antioxidant enzymes and decreasing toxic products such as lipid peroxidation by products.[29]

CONCLUSION

Aqueous extract from stem bark of *Albizia gummifera* exhibited strong antisalmonellal and antioxidant activity in different *in vivo* systems. Further work is necessary to isolate active principles and elucidate the actual mechanism involved in the antioxidant activity of this plant.

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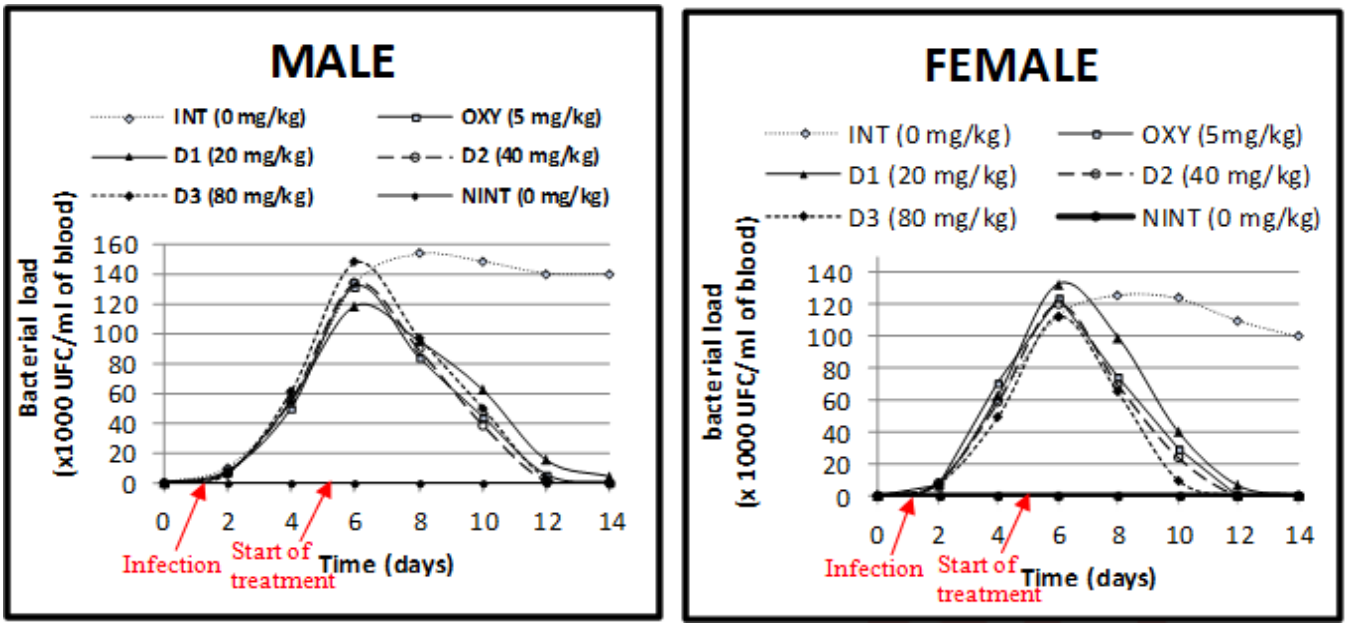

INT: Infected and untreated; NINT: Uninfected and untreated; OXY: Oxytetracyclin; D: Dose of plant extract.

Figure: Effect of the *A. gummifera* extract on rats salmonella's blood load.

Table 1. Evolution of the organs and serum catalase activity with different treatments

Doses (mg/kg bw)	Quantity of catalase (mmol/min/g of tissues and mmol/min/ml of blood)					
	Serum	Liver	Lungs	Spleen	Heart	Kidneys
Male						
INT(0)	0.71±0.01^{a}	0.87±0.04^{a}	0.89±0.08^{a}	0.68±0.01^{a}	0.95±0.05^{a}	1.71±0.07^{a}
OXY(5)	0.93±0.05b^{c}	1.76±0.24^{b}	1.49±0.37^{b}	0.96±0.10^{bc}	1.50±0.13^{cd}	3.27±0.37^{bc}
NINT(0)	0.98±0.03^{c}	1.41±0.07^{bc}	1.62±0.13^{b}	1.06±0.00^{cd}	1.42±0.01^{bc}	3.60±0.10^{bc}
D1 (20)	0.85±0.04^{b}	1.63±0.49^{bc}	1.52±0.34^{b}	0.89±0.03^{b}	1.28±0.07^{b}	3.15±0.50^{b}
D2 (40)	0.92±0.08b^{c}	1.94±0.13^{cd}	1.79±0.31^{bc}	1.08±0.01^{d}	1.46±0.03^{cd}	3.47±0.39^{bc}
D3 (80)	0.96±0.03^{c}	2.33±0.18^{d}	2.24±0.15^{c}	1.13±0.08^{d}	1.57±0.06^{d}	3.84±0.02^{c}
Female						
INT(0)	0.75±0.16^{a}	1.36±0.05^{a}	1.14±0.09^{a}	1.08±0.11^{a}	0.98±0.13^{a}	1.60±0.31^{a}
OXY(5)	0.75±0.15^{a}	1.38±0.06^{ab}	1.44±0.37^{a}	1.53±0.35^{ab}	1.20±0.05^{b}	1.72±0.35^{a}
NINT(0)	0.79±0.02^{a}	1.64±0.15^{b}	1.43±0.08^{a}	1.50±0.28^{ab}	1.31±0.01^{b}	1.80±0.05^{a}
D1 (20)	0.78±0.09^{a}	1.51±0.08^{ab}	1.32±0.09^{a}	1.43±0.06^{ab}	1.23±0.07^{b}	1.74±0.11^{a}
D2 (40)	0.78±0.01^{a}	1.53±0.11^{ab}	1.46±0.15^{a}	1.51±0.28^{ab}	1.32±0.05^{b}	1.76±0.04^{a}
D3 (80)	0.77±0.00^{a}	1.59±0.00^{ab}	1.41±0.00^{a}	1.64±0.23^{b}	1.32±0.04^{b}	1.85±0.08^{a}

Data are expressed as mean ± S.E.M. n = 4. Values for a given group in a line followed by same letter as superscript are not significantly different according to Waller Duncan's multiple comparison test ($P < 0.05$). INT: Infected and untreated; NINT: Uninfected and untreated; OXY: Oxytetracyclin; D: Dose of plant extract.

Table 2. Evolution of the organs and serum peroxidase activity with different treatments.

Doses (mg/kg bw)	Quantity of peroxidase (µmol/min/g of tissues and µmol/min/ml of blood)					
	Serum	Liver	Lungs	Spleen	Heart	Kidneys
Male						
INT(0)	52.37±8.58^{a}	146.57±25.25^{a}	187.20±52.16^{a}	534.43±50.31^{a}	136.11±25.44^{a}	288.73±27.60^{a}
OXY(5)	53.04±4.51^{a}	210.47±35.61^{ab}	286.87±3.90^{bc}	693.80±72.47^{b}	186.67±25.37^{ab}	430.30±27.16^{b}
NINT(0)	58.85±5.01^{a}	230.37±25.73^{bc}	320.87±58.22^{cd}	804.90±38.81^{b}	228.40±0.50^{b}	467.87±33.80^{b}
D1 (20)	53.97±0.68^{a}	229.23±12.78^{bc}	241.17±32.08^{ab}	701.60±15.74^{b}	190.93±92.54^{ab}	456.3±02.68^{b}
D2 (40)	57.20±4.00^{a}	243.47±67.68^{bc}	272.93±35.76b^{c}	770.83±80.25^{b}	199.47±15.56^{ab}	468.37±55.04^{b}
D3 (80)	57.23±3.12^{a}	285.37±21.26^{c}	374.13±19.54^{c}	725.20±52.02^{b}	245.47±9.64^{b}	463.07±8.38^{b}
Female						
INT(0)	12.09±1.90^{a}	429.50±86.08^{a}	279.53±63.73^{a}	1588.00±74.8^{7a}	444.13±50.10^{a}	541.80±54.89^{a}
OXY(5)	19.70±0.70^{b}	498.30±31.53^{a}	356.73±33.96^{a}	1997.67±103.36^{bcd}	817.37±21.89^{b}	634.93±50.55^{a}
NINT(0)	19.48±0.64^{b}	542.23±11.32^{a}	396.20±25.34^{a}	1903.67±136.58^{ab}	1012.73±82.04^{c}	629.60±67.67^{a}
D1 (20)	18.05±2.72^{b}	509.83±53.70^{a}	376.70±80.20^{a}	1798.00±84.18^{abc}	852.83±12.60^{b}	508.93±64.69^{a}
D2 (40)	18.55±2.36^{b}	530.27±40.83^{a}	397.10±93.58^{a}	2246.33±20.81^{d}	1004.53±42.83^{c}	534.70±31.45^{a}
D3 (80)	18.92±0.06^{b}	528.03±33.83^{a}	372.73±14.61^{a}	2235.67±157.15^{cd}	965.30±63.56^{c}	560.70±0.17^{a}

Data are expressed as mean ± S.E.M. n = 4. Values for a given group in a line followed by same letter as superscript are not significantly different according to Waller Duncan's multiple comparison test ($P < 0.05$). INT: Infected and untreated; NINT: Uninfected and untreated; OXY: Oxytetracyclin; D: Dose of plant extract.

Table 3. Evolution of the organs and serum nitroxide (NO) antioxidant activity with different treatments

Doses (mg/kg bw)	Quantity of NO (µmole/g of tissues and µmole/ml of blood)					
	Serum	Liver	Lungs	Spleen	Heart	Kidneys
Male						
INT(0)	5991.63±348.56^{d}	938.27±138.99^{b}	2434.67±342.95^{b}	3064.67±282.91^{b}	708.10±36.86^{a}	2748.00±12.16^{a}
OXY(5)	3909.57±47.40^{ab}	852.17±67.06^{ab}	2079.00±119.85^{ab}	2805.00±45.03^{b}	610.87±57.69^{a}	2589.67±196.29^{a}
NINT(0)	3864.30±455.93^{a}	908.00±83.803^{ab}	2076.67±219.40^{ab}	2152.33±162.54^{a}	634.07±52.94^{a}	2512.00±387.93^{a}
D1 (20)	5507.97±479.75^{cd}	927.50±121.515^{ab}	2112.67±223.75^{ab}	2726.33±172.08^{a}	634.77±47.01^{a}	2472.67±39.46^{a}
D2 (40)	5206.77±780.75^{cd}	740.13±6.217^{a}	2023.00±32.23^{ab}	2321.00±61.65^{a}	568.53±71.84^{a}	2558.33±254.50^{a}
D3 (80)	4783.70±290.74^{bc}	826.57±16.865^{ab}	1972.33±154.84^{a}	2089.67±198.50^{a}	657.10±91.70^{a}	2537.00±35.79^{a}
Female						
INT(0)	2414.00±134.62^{a}	2598.33±262.42^{a}	4163.00±400.14^{a}	8974.00±262.77^{a}	2225.33±375.92^{a}	6553.67±410.26^{a}
OXY(5)	1918.27±396.10^{a}	2052.67±175.82^{a}	3957.00±148.33^{a}	8057.67±374.13^{a}	1941.00±407.02^{a}	6371.33±526.36^{a}
NINT(0)	1821.27±130.65^{a}	2101.67±95.03^{a}	3693.67±270.64^{a}	7808.00±77.67^{a}	1952.67±541.42^{a}	6277.00±113.22^{a}
D1 (20)	1839.27±329.42^{a}	2119.33±95.03^{a}	3721.67±360.76^{a}	8045.33±362.50^{a}	2088.00±401.16^{a}	6452.67±30.82^{a}
D2 (40)	1921.83±192.89^{a}	2131.33±180.62^{a}	3617.00±409.36^{a}	7735.33±206.53^{a}	2086.00±298.87^{a}	6290.33±462.73^{a}
D3 (80)	1990.10±423.14^{a}	2048.67±30.60^{a}	3536.67±442.25^{a}	7821.67±391.41^{a}	1927.33±315.45^{a}	6340.33±312.34^{a}

Data are expressed as mean ± S.E.M. n = 4. Values for a given group in a line followed by same letter as superscript are not significantly different according to Waller Duncan's multiple comparison test ($P < 0.05$). INT: Infected and untreated; NINT: Uninfected and untreated; OXY: Oxytetracyclin; D: Dose of plant extract.

Table 4. Evolution of the Organs and serum MDA with different treatments

Doses (mg/kg bw)	Quantity of MDA (µM/ml of blood and µM/g of tissues)					
	Serum	Liver	Lungs	Spleen	Heart	Kidneys
Male						
INT(0)	0.022±0.002^{d}	0160±0020^{a}	0.138±0.041^{a}	0.152±0.020^{b}	0.097±0001^{d}	0.141±0.003^{d}
OXY(5)	0.012±0.001^{ab}	0.131±0.024^{a}	0.119±0.015^{a}	0.135 ±0.004^{ab}	0.077±0003.^{b}	0.125±0.001 ^{c}
NINT(0)	0.013±0.001^{bc}	0.148±0.000^{a}	0.133±0.040.^{a}	0.125±0.025^{ab}	0.063±0001^{a}	0.112±0.005 ^{a}
D1 (20)	0.015±0.001^{c}	0.152±0.027^{a}	0.134±0.024^{a}	0.139 ±0.013^{ab}	0.088±0003^{c}	0.121±0.003^{bc}
D2 (40)	0.010±0.001^{a}	0.147±0.016^{a}	0.127±0.010^{a}	0.012±0.014.^{ab}	0.058±0009^{a}	0.117±0.002^{ab}
D3 (80)	0.011±0.002^{a}	0.133±0.006 ^{a}	0.117±0.015^{a}	0.117±0.002^{a}	0.058 ±0002^{a}	0.116±0.002^{ab}
Female						
INT(0)	0.015±0.001^{a}	0.115±0.010^{d}	0.050±0.002^{d}	0.056±0.004^{d}	0.041±0.003^{c}	0.057±0.007^{c}
OXY(5)	0.012±0.000^{a}	0.069±0.014^{b}	0.043±0.001^{cd}	0.050±0.001^{bc}	0.040±0.003^{bc}	0.053±0.002^{bc}
NINT(0)	0.012±0.002^{a}	0.043±0.001^{a}	0.035±0.003^{a}	0.047±0.003^{ab}	0.034±0.002^{abc}	0.043±0.004^{ab}
D1 (20)	0.014±0.000^{a}	0.089±0.004^{c}	0.042±0.003^{ab}	0.043±0.006^{ab}	0.037±0.001^{abc}	0.041±0.001^{ab}
D2 (40)	0.012±0.000^{a}	0.088±0.004^{c}	0.040±0.001^{ab}	0.043±0.002^{ab}	0.028±0.002^{ab}	0.036±0.003^{a}
D3 (80)	0.013±0.001^{a}	0.078±0.002^{bc}	0.045±0.001^{cd}	0.042±0.000^{a}	0.026±0.002^{a}	0.036±0.000^{a}

Data are expressed as mean ± S.E.M. n = 4. Values for a given group in a line followed by same letter as superscript are not significantly different according to Waller Duncan's multiple comparison test ($P < 0.05$). INT: Infected and untreated; NINT: Uninfected and untreated; OXY: Oxytetracyclin; D: Dose of plant extract.

Table 5. Effect of treatment with different doses of *A. gummifera* extract on rat's alkaline phosphatase (ALP) and bilirubin.

Doses (mg/kg bw)	ALP (UI/L)	Bilirubin (mg/dl)	
		Total	Direct
Male			
INT(0)	301,47±3,92^{c}	0,50±0,00^{b}	0,40±0,12^{c}
OXY(5)	258,85±23,95^{bc}	0,32±0,05^{a}	0,25±0,01^{a}
NINT(0)	140,53±19,29^{a}	0,38±0,05^{a}	0,28±0,01^{b}
D1 (20)	163,83±2,99^{a}	0,45±0,00^{b}	0,22±0,01^{a}
D2 (40)	149,01±24,64^{a}	0,32±0,00^{a}	0,23±0,02^{a}
D3 (80)	221,68±3,84^{b}	0,35±0,01^{a}	0,23±0,01^{a}
Female			
INT(0)	404,83±7,96^{b}	0,38±0,03^{c}	0, 32±0,02^{b}
OXY(5)	173,63±14,66^{a}	0,32±0,02^{bc}	0,31±0,02^{b}
NINT(0)	136,91±13,69^{a}	0,24±0,01^{a}	0,23±0,00^{a}
D1 (20)	167,28±10,79^{a}	0,36±0,03^{bc}	0,30±0,03^{b}
D2 (40)	150,51±36,22^{a}	0,30±0,00^{ab}	0,29±0,01^{b}
D3 (80)	171,36±13,11^{a}	0,26±0,04^{a}	0,25±0,01^{a}

Data are expressed as mean ± S.E.M. n = 4. Values for a given group in a line followed by same letter as superscript are not significantly different according to Waller Duncan's multiple comparison test ($P < 0.05$). INT: Infected and untreated; NINT: Uninfected and untreated; OXY: Oxytetracyclin; D: Dose of plant extract

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