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Effect of *Salmonella typhimurium* infection on rat's cell oxidation and *in vivo* antioxidant activity of *Vitellaria paradoxa* and *Ludwigia abyssinica* aqueous extract

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Comments

By doing this work these authors have contributed to the development of alternatives drug from natural origin with positive synergetic effect and can therefore be recommended for the treatment of typhoid fever and for the restoration of cell redox equilibrium during bacterial infection.

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ABSTRACT

Objective: To evaluate the effect of bacterial infection on cell oxidation and to study the *in vitro* and *in vivo* antioxidant activity of aqueous extract of *Ludwigia abyssinica* and *Vitellaria paradoxa*.

Methods: The *Salmonella typhimurium*-infected rats (1.5×10^8 CFU) concurrently received either different doses of plant extract (55, 110, 220 and 440 mg/kg) or ascorbic acid (vitamin C 100 mg/kg) daily for 18 d. The parameters like, lipid profile, reduced glutathione content, superoxide dismutase, catalase activities and bilirubin were assessed.

Results: Infection has resulted in an increase of heart reduced glutathione, heart and kidneys malondihaldehyde and liver superoxide dismutase activity followed by decreases of that of heart. The administration of the extract at 55, 110, 220 and 440 mg/kg body has resulted in the correction of some of these injuries.

Conclusions: The present study demonstrates that aqueous extract of *Ludwigia abyssinica* and *Vitellaria paradoxa* can fight against bacterial infection and cell oxidation induced by infection with *Salmonella typhimurium*.

KEYWORDS

Oxidative stress, *Ludwigia abyssinica*, *Vitellaria paradoxa*, *Salmonella typhimurium*, Bacteria infection

1. Introduction

Recent studies show that free radicals affect the biological systems which lead to different cell damages[1], while interest in natural antioxidants in relation to their therapeutic properties has also significantly increased. The

antioxidant activity of a compound is its ability to directly prevent or inhibit cells oxidation. Although a variety of intrinsic antioxidant are present in the organism [superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT) and peroxidase] which could also attenuate oxidative damage of a tissue indirectly by enhancing natural defenses of cell and/or

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directly by scavenging the free radical species^[2–4], infectious diseases lead to the malfunction of some of these antioxidant systems, thereby over production of oxygen reactive species. This result in cell damage leads to the pathogenesis of a variety of human diseases^[5,6]. Many synthetic antioxidants have been used (butylated–hydroxyanisole, butylated–hydroxytoluene and tert–butylhydroquinone) but they are now restricted because of their toxic effects. The efforts for the development of alternative antioxidants of natural origin have received much attention in the last decade and appeared to be favourable in many respects as compared to chemical antioxidant^[7].

Ludwigia abyssinica (*L. abyssinica*) A. Rich. belongs to the family of Onograceae that is widely distributed in the tropical region. It predominantly contains aquatic herbs and shrubs growing in ditches, shallow and marshy areas, riverbanks, ponds and slow moving streams^[8]. Leaves of *L. abyssinica* are used in Nigeria as vegetables and previous studies on several species have revealed it to be of value in traditional medicine^[8]. Antimicrobial (antibacterial and antifungal) activity of many *Ludwigia* spp. (*Ludwigia adscendens*, *Ludwigia hyssopifolia*, *L. abyssinica* and *Ludwigia octovalvis*, *Ludwigia decurrens*) has been shown by Oyedeji et al. and Das et al^[8,9]. Apart from this antimicrobial activity, antioxidant properties of other *Ludwigia* spp. especially *Ludwigia octovalvis* has been shown by Shyur et al^[10].

Vitellaria paradoxa (*V. paradoxa*), commonly known as shea butter tree, belongs to Sapotaceae family and is largely distributed to the semi–arid zone of sub–Saharan Africa from Senegal in the west to Uganda^[11]. Shea butter has long been used in sub–Saharan Africa for medicinal, culinary and other applications and served as a cocoa butter equivalent in the manufacture of chocolate as well as an ingredient in cosmetics^[12]. It is the main edible oil for the people of Northern Ghana and much of Western Africa and the most important source of fatty acids and glycerol in their diet^[12]. Despite being increasingly replaced by commercially produced lotions in many communities, shea butter is traditionally used as a skin and hair moisturizer and for the protection against the sun^[13]. It is also a kind of valuable medicine to relieve rheumatic and joint pains and is applied to open wounds to quicken healing times and prevent infection.

The antifungal activity of aqueous and ethanol extracts of the plant bark^[14] as well as the antibacterial activity of molecules isolated from its root bark have recently been reported by Garba and Salihu^[15]. In the West Region of Cameroon, this plant (leaves and stem bark) is used for the treatment of skin diseases, fever and rheumatism. Its leaves and bark infusion and decoction are respectively used with other medicinal plants for the treatment of urinogenital infections, micro filarial cutaneous infections and typhoid fever. Although the *in vitro* antimicrobial and antioxidant activity of these plants has been shown, there are no scientific work neither on their separated *in vivo* activity nor

that on their mixture. Keeping on viewing the traditional uses and previous work done *in vitro* on these plants, the present study was then undertaken to evaluate the *in vitro* and *in vivo* antioxidant activity of *L. abyssinica* and *V. paradoxa* aqueous extract on *Salmonella typhimurium* (*S. typhimurium*) infected rats.

2. Material and methods

2.1. Plant material and extract preparation

L. abyssinica (total aerial part) and *V. paradoxa* leaves were collected from field–grown plants at the vegetative stage in Noun Sub Division (West Region of Cameroon), then identified at the Cameroon National Herbarium where the voucher specimen is deposited under the reference number 25403/HNC and 50216/HNC, respectively.

They were then dried at room temperature and ground to a fine powder. The extracts were prepared as earlier described by Moroh et al.^[16] with slight modification. A total of 1 L of boiling distilled water was poured into 100 g of plant powder under constant shaking with magnetic stirrer for 10 min. At the end of the extraction, it was filtered through nylon mesh followed by Whatman filter paper No. 1 (Whatman Ltd., Germany). The filtrate was then concentrated under reduced pressure on rotary evaporator at 40 °C and the extract obtained was kept frozen (–18 °C) until further use.

2.2. Animal treatment

In this study, 60 mature rats (aged between 8 to 9 weeks) were used. The animals were distributed randomly into seven groups of four animals each with similar average body weight on the third day after immunosuppression. Apart from animals of group 1, those of all other groups (2–7) were infected. Group 1 served as a neutral control. Animals of group 2 received orally 1 mL of a suspension containing 1.5×10^8 CFU of *S. typhimurium* on the third day of immunosuppression. They were not subsequently treated and thus served as negative control. Group 3 animals received the infection of 40 mg/kg oxytetracyclin (positive control) everyday from the seventh day. Animals of other groups (4–7) were treated from the seventh day after infection with different doses of the plant extract (55, 110, 220 and 440 mg/kg body weight). During the treatment, experimental animals were treated in accordance with 1986, 86/609/EEC European Community guidelines for laboratory animal use and care.

2.3. Preparation of homogenates

The homogenates of various organs were prepared at 15% in 0.15 mol/L phosphate buffer (pH 7.4). This was done by grinding 0.6 g of organ in 4 mL of buffer. After centrifugation at 3000 r/min for 15 min, the supernatant was taken and kept

frozen until further use.

2.4. Evaluation of biochemical parameters

Prior to sacrifice, animals were subjected to a 12-hour food fasting and were anesthetized by injection of ketamine/Valium solution (4/1) and dissected. Their blood were collected by abdominal artery puncture and fed into sterilized test tubes. It was then allowed to stand for 30 min before being centrifuged at 3000 r/min for 10 min. Organs such as heart, liver, spleen and kidneys were removed. Sera and organs homogenates were used for the determination of biochemical parameters related to oxidative stress such as reduced GSH, SOD, malondialdehyde (MDA), nitric oxide (NO) and lipid profile (LP).

2.5. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The *in vitro* antioxidant activity were determined through the capacity of the extract to reduce DPPH with modified method of Obeid *et al.*[17]. DPPH solution was prepared by dissolving 3.92 mg in 100 mL of methanol. The different concentrations of extract were 31.2, 62.5, 125, 250, 500, 750, 1000, 2000 µg/mL while those of ascorbic acid (positive control) were 12.5, 25, 50, 75, 100, 150, 200 µg/mL. A test sample solution in methanol (1 mL) was added to 1.5 mL of DPPH methanol solution. After shaking, the mixture was incubated for 10 min in darkness at room temperature and then absorbance was measured at 517 nm. The difference in absorbance between the test samples and control (methanol) was taken as the activity. The activity, expressed as IC₅₀ value (µg/mL: 50% inhibitory concentration) was consistent with the mean value of 3 measurements. The inhibitory effect of DPPH was calculated according to the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The radical scavenging activity was expressed in terms of IC₅₀ calculated by the graphic method.

2.5.1. SOD activity

SOD activity was determined in tissue by Fabio *et al.*[18] method with some modifications. To 150 µL of homogenates 1 650 µL of phosphate buffer (pH 7.2) and 200 µL of 0.3 mmol/L epinephrine was also added. The self-oxidation of epinephrine was recorded at 480 nm 30 seconds and then, one minute after its addition by spectrophotometer (Schimadzu 1501, Japan). The SOD activity expressed as percentage of inhibition was calculated taking into account that 50% inhibition corresponds to one unit of activity.

2.5.2. CAT activity

CAT activity was determined in tissue by Tozan *et al.*[19] method with some modifications. A total of 50 µL of the homogenates was added to tubes containing 750 µL of

phosphate buffer (pH 7.2) and 200 µL of 50 mmol/L H₂O₂. After one minute incubation at room temperature, 2 mL of dichromate was added. The mixture was homogenated and incubated at 100 °C for 10 min then cooled in ice bath and the absorbance was recorded at 570 nm using Shimadzu 1501, Japan spectrophotometer. One unit of activity is equal to one mmol/L of H₂O₂ degraded per minute and is expressed as units per milligram of protein.

Lipid peroxidation (LPO) activity: LPO was determined in tissue by Oyedemi *et al.*[7] method with some modifications. A total of 0.5 mL of 1% orthophosphoric acid and 0.5 mL of precipitating mixture (1% thiobarbituric acid, 1% acetic acid) were added to 0.1 mL of homogenate. The mixture was homogenated and heated in boiling water for 15 min and cooled immediately. It was then centrifuged at 5000 r/min for 10 min and the absorbance of the supernatant was recorded at 532 nm using Shimadzu 1501, Japan spectrophotometer. The LPO was calculated based on the molar extinction coefficient of MDA (153 L/mmol-cm), and expressed in terms of micromoles of MDA/g of tissue.

2.5.3. Reduced GSH activity

GSH was determined in tissue by Oyedemi *et al.*[7] method with some modifications. A total of 0.8 mL of 0.3 mol/L dihydrate sodium phosphate solution was added to 0.2 mL of homogenate. It was centrifuged at 5000 r/min for 5 min and 0.5 mL of 0.4 mg/mL dithiobis-nitrobenzoate (prepared in 1% sodium citrate) was added to the supernatant. The optical density was recorded at 412 nm. The total GSH was calculated based on the molar extinction coefficient of GSH (1.36×10⁵ L/mmol-cm) and expressed in terms of micromoles of GSH/g of tissue.

2.5.4. Statistical analysis

Data obtained were expressed as mean±SEM (Standard Error of Mean) and were analysed using One way ANOVA. Duncan test was used to compare means of different groups. A probability value less than 0.05 was considered statistically significant.

3. Results

3.1. *In vivo* antityphoid properties and side effects of the extract

The evolution of the bacterial load in the faeces of experimental rats throughout the experiment is summarized in Figures 1 to 3 below. From the second to the sixth day after infection, the bacterial load continuously increased in the faeces of infected animals. The healing effect of *L. abyssinica* extract was observed from the ninth day (the third day of treatment) since there was a significant and dose-dependent decrease of bacterial load in infected animals under treatment. Similarly, there was a slight decrease in

bacterial load in negative control group animals but the load remained relatively high on the last day of treatment (1.93×10^6) as compared to that of animals receiving different doses of extract which were healed after 12, 16 and 18 d of treatment respectively with different doses of *V. paradoxa*, *L. abyssinica* and the mixture.

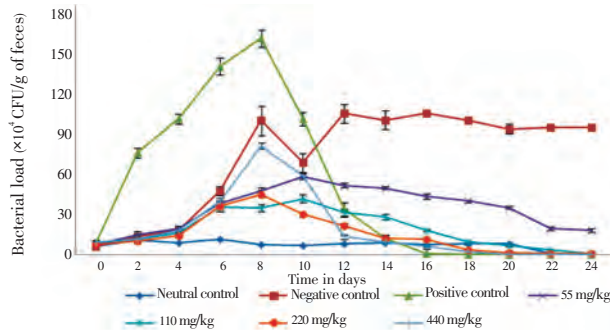


Figure 1. Effect of the *V. paradoxa* extract on rats *Salmonella*'s feces load.

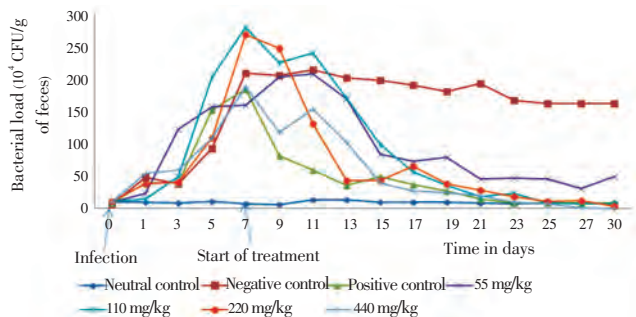


Figure 2. Effect of the *L. abyssinica* extract on rats *Salmonella*'s feces load.

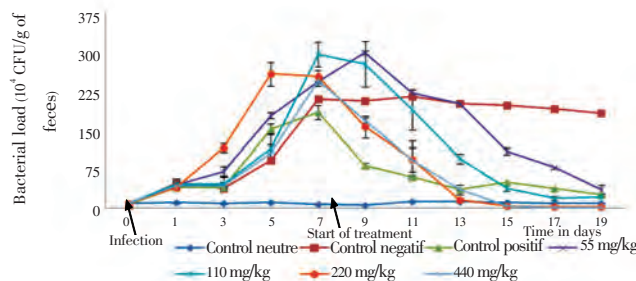


Figure 3. Effect of the mixture extract on rats *Salmonella*'s feces load.

3.2. DPPH radical-scavenging activity

Antioxidative activity of different extracts were measured by DPPH free radical scavenging method (Table 1) and their scavenging activities were compared with the standard antioxidant ascorbic acid. Both ascorbic acid and extracts showed dose dependent activities. However, the DPPH free radical scavenging effect of plant extract was smaller than that of ascorbic acid. Percent (%) scavenging activity or % inhibition was plotted against log concentration and from the graph IC_{50} (inhibition concentration 50) value was calculated by linear regression analysis. IC_{50} values were found to be 141.78 $\mu\text{g/mL}$, 320.03 $\mu\text{g/mL}$, 259.83 and 59.31 $\mu\text{g/mL}$ respectively, for *L. abyssinica*, *V. paradoxa*, the mixture and ascorbic acid (Table 1).

Table 1

DPPH scavenging activity of different plant extracts (*L. abyssinica*, *V. paradoxa* and their mixture) and ascorbic acid.

Test material	<i>L. abyssinica</i>	<i>V. paradoxa</i>	Mixture	Ascorbic acid	
log conc	% inhibition	% inhibition	% inhibition	% inhibition	log conc
3.30	94.00	81.00	86.00	97.00	2.30
3.00	86.00	81.00	85.00	85.00	2.17
2.87	75.00	70.00	71.00	77.00	2.00
2.69	63.00	51.00	58.00	55.00	1.87
2.39	57.00	49.00	53.00	33.00	1.69
2.09	49.00	26.00	32.00	7.00	1.39
1.79	40.00	17.00	20.00	3.00	1.09
IC_{50} (μg)	141.78	320.03	259.83	59.31	

3.3. Effect of treatment on heart and liver GSH

Figures 4 and 5 below show the effect of treatment on heart and liver GSH. It follows from the analysis of these figure that, infection resulted in a significant increase in heart and liver GSH. With the exception of the significant decrease observed in animals receiving the extract of *L. abyssinica* at 440 mg/kg compared to the negative control, the increase in heart GSH persisted with the administration of various doses of *V. paradoxa* as well as their mixture. The rate of liver GSH has not undergone any significant changes during treatment (Figure 5).

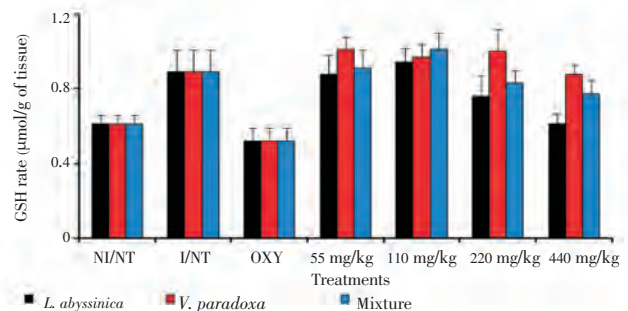


Figure 4. Effect of treatment on heart GSH.

NI/NT=No infected/No treated group, I/NT=Infected/No treated group, OXY=Oxytetracyclin.

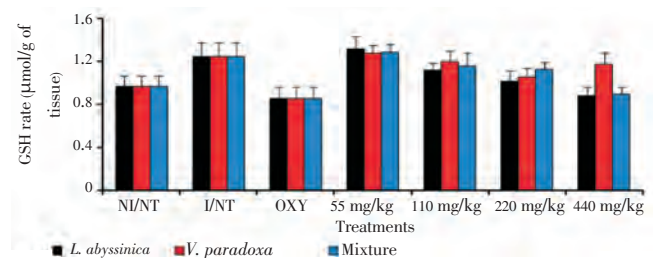


Figure 5. Effect of treatment on liver GSH.

NI/NT=No infected/No treated group, I/NT=Infected/No treated group, OXY=Oxytetracyclin.

3.4. Effect of treatment on heart and kidney MDA

Figures 6 and 7 below show that infection caused a significant increase in heart and kidney MDA. Even though not significant, the administration of different extracts at

different doses resulted in a dose dependent decrease in the rate of heart (Figure 6) and kidney (Figure 7) MDA compared to the negative control.

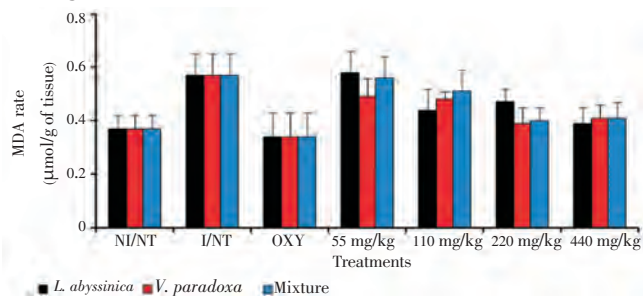


Figure 6. Effect of different treatments on heart MDA.

NI/NT=No infected/No treated group, I/NT=Infected/No treated group, OXY=Oxytetracyclin.

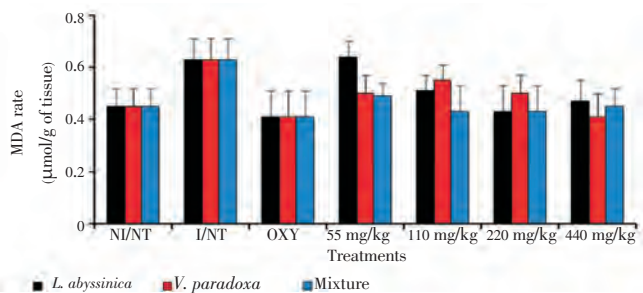


Figure 7. Effect of different treatments on kidney MDA.

NI/NT=No infected/No treated group, I/NT=Infected/No treated group, OXY=Oxytetracyclin.

3.5. Effect of treatment on rate's kidney NO

As illustrated by Figure 8 below, infection resulted in an increase in the rate of kidney NO compared to neutral control. This increase persisted with the administration of different doses of *V. paradoxa* extract, whereas the other extracts (*L. abyssinica* and the mixture), administered at doses level 220 and 440 mg/kg resulted in non-significant decrease of kidney NO as compared to negative control.

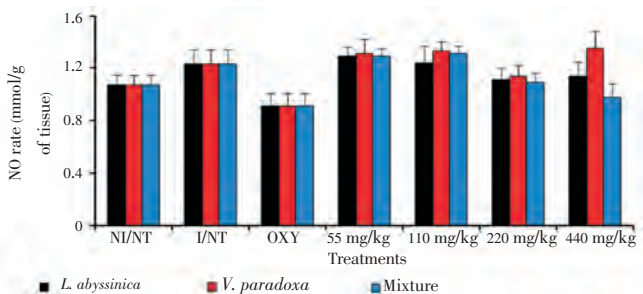


Figure 8. Effect of different treatments on kidney NO level.

NI/NT=No infected/No treated group, I/NT=Infected/No treated group, OXY=Oxytetracyclin.

3.6. Effect of treatment on heart, liver and kidney SOD activity

As shown in Figures 9 to 11 below revealed that, infection resulted in significant decrease in heart and kidney SOD activity compared to the neutral control. Apart from these variations, no significant change was observed with the different treatments compared with controls.

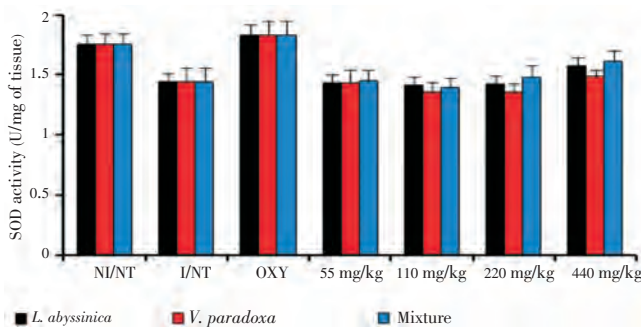


Figure 9. Effect of different treatments on heart SOD activity.

NI/NT=No infected/No treated group, I/NT=Infected/No treated group, OXY=Oxytetracyclin.

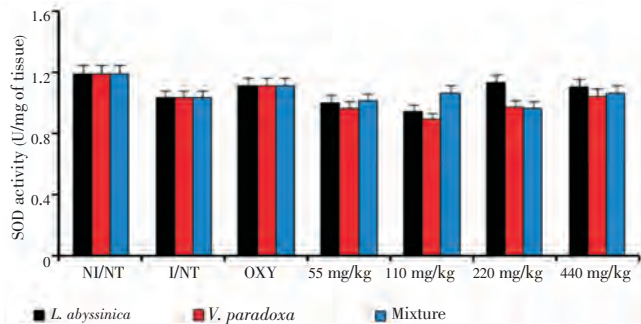


Figure 10. Effect of different treatments on kidney SOD activity.

NI/NT=No infected/No treated group, I/NT=Infected/No treated group, OXY=Oxytetracyclin.

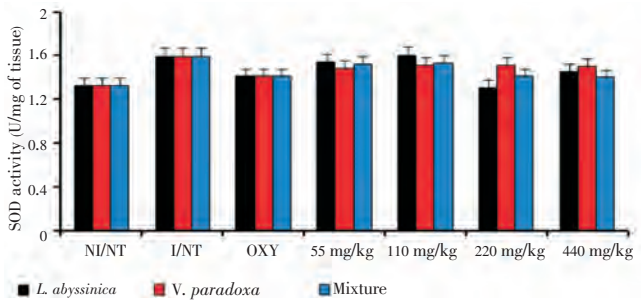


Figure 11. Effect of different treatments on liver SOD activity.

NI/NT=No infected/No treated group, I/NT=Infected/No treated group, OXY=Oxytetracyclin.

3.7. Effect of treatment on liver heart and kidney CAT activity

The evolution of the activity of the liver, heart and kidney CAT with different treatments is shown respectively in Figures 12 to 14 below. It is clear from these figures that the infection resulted in a significant decrease in the activity of hepatic and cardiac CAT, whereas renal CAT activity has not significantly changed as compared to neutral control.

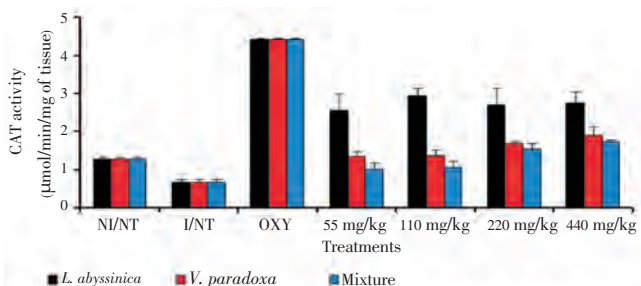


Figure 12. Effect of different treatments on liver CAT activity.

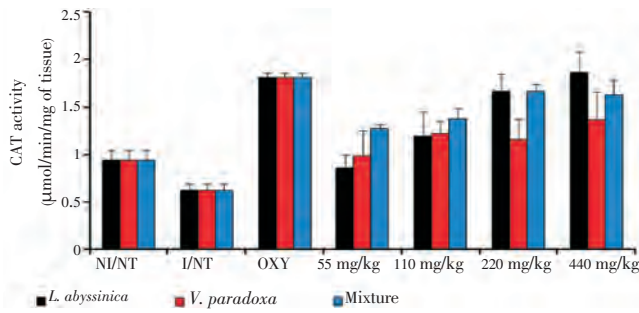


Figure 13. Effect of different treatments on heart CAT activity.

Figure 12 shows an increase in liver CAT activity in animals treated with different doses *V. paradoxa* extract, while other treatments have resulted in a non-significant changes in the activity of liver CAT compared to neutral control. However, as compared to the negative control, liver CAT activity has significant increase in animals treated with *L. abyssinica* extracts and mixture at doses ≥ 110 mg/kg.

Figure 13 in turn shows that the administration of different doses of extract caused a dose-dependent increase in heart CAT relative to the negative control. Regarding kidney CAT (Figure 14), their activity were significantly increased in animals treated with the extract of *V. paradoxa* at doses ≥ 220 mg/kg.

The infection resulted in no significant change in the activity of renal CAT compared to neutral control (Figure 14), but the administration of various extracts resulted in a dose-dependent increase in renal CAT compared to controls.

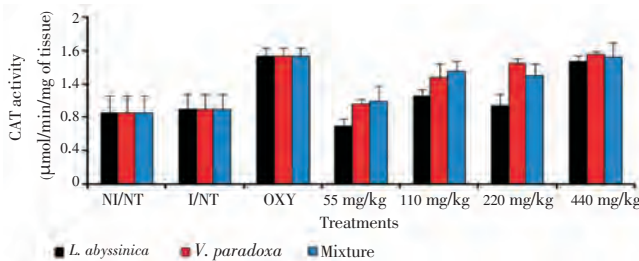


Figure 14. Effect of different treatments on kidney CAT activity.

3.8. Effect of treatment on LP

The effect of treatment on the LP of test animals is summarized in Tables 2 to 4 below.

Table 2

Effect of treatment with different doses of *L. abyssinica* extract on rat’s LP.

Doses of extract (mg/kg)	Total cholesterol	HDL	LDL	Triglycerides	Arterogenic index
Ui/Ut	113.57±6.09 ^a	45.89±3.13 ^a	48.42±4.68 ^a	95.80±6.08 ^a	1.47±0.95 ^a
I/Ut	133.20 ±4.76 ^b	47.66 ±2.45 ^a	68.74±3.05 ^b	83.98 ±7.86 ^a	1.79±0.96 ^a
55 mg/kg	134.53±5.04 ^b	46.35±3.41 ^a	71.69±5.22 ^b	82.43±4.23 ^a	1.90±0.70 ^a
110 mg/kg	145.92±5.31 ^b	47.76±4.01 ^a	79.90±4.18 ^b	91.27±4.80 ^a	2.05±0.64 ^a
220 mg/kg	136.67±3.59 ^b	49.90±2.29 ^a	68.74±4.27 ^b	90.14±5.12 ^a	1.73±0.82 ^a
440 mg/kg	141.46±4.23 ^b	52.83±5.47 ^a	71.61±3.07 ^b	85.09±6.08 ^a	1.67±0.84 ^a

Ui/Ut: Uninfected and untreated; I/Ut: Infected and untreated. ^{a,b,c}Values are mean±SEM of four trials. Values on the same column with the same letters are not significantly different at $P>0.05$.

It appears that, although infection and treatment resulted in no significant change in high density lipoprotein (HDL) cholesterol, triglycerides and arteriosclerosis index, infection has resulted in a significant increase of total and low density

lipoprotein (LDL) cholesterol as compared to the neutral control. Apart from the significant increase in total and LDL cholesterol level in animals treated with different doses of *V. paradoxa* as compared to neutral and negative controls, these parameters were not significantly affected by administration of different doses of *L. abyssinica* and their mixture.

Table 3

Effect of treatment with different doses of *V. paradoxa* extract on rat’s LP.

Doses of extract (mg/kg)	Total cholesterol	HDL	LDL	Triglycerides	Arterogenic index
Ui/Ut	113.57±6.09 ^a	45.89±3.13 ^a	48.52±4.68 ^a	95.80±6.08 ^a	1.47±0.95 ^a
I/Ut	133.20±4.76 ^b	47.66±2.45 ^a	68.74±3.05 ^b	83.98±7.86 ^a	1.79±0.96 ^a
55	153.35±6.29 ^c	47.30±3.36 ^a	89.16±4.92 ^c	84.45±6.12 ^a	2.24±0.61 ^a
110	155.72±6.91 ^c	47.43±3.40 ^a	91.39±5.11 ^c	84.50±5.27 ^a	2.28±0.52 ^a
220	151.13±8.81 ^c	52.50±5.15 ^a	80.75±3.66 ^d	89.38±5.11 ^a	1.87±0.48 ^a
440	149.42±7.27 ^c	49.18±3.51 ^a	82.00±2.50 ^{cd}	91.17±6.05 ^a	2.04±0.49 ^a

Ui/Ut: Uninfected and untreated; I/Ut: Infected and untreated. ^{a,b,c}Values are mean±SEM of four trials. Values on the same column with the same letters are not significantly different at $P>0.05$.

Table 4

Effect of treatment with different doses of mixture on rat’s LP.

Doses of extract (mg/kg)	Total cholesterol	HDL	LDL	Triglycerides	Arterogenic index
Ui/Ut	113.57±6.09 ^a	45.89±3.13 ^a	48.52±4.68 ^a	95.80±6.08 ^a	1.47±0.95 ^a
I/Ut	133.20 ±4.76	47.66±2.45 ^a	68.74±3.05 ^b	83.98±7.86 ^a	1.79±0.96 ^a
55	135.41±4.61 ^b	48.08±3.18 ^a	70.69±4.17 ^b	83.18±3.60 ^a	1.81±0.55 ^a
110	134.20±5.22 ^b	46.83±3.75 ^a	71.07±3.03 ^b	81.49±4.19 ^a	1.86±0.75 ^a
220	138.07±5.02 ^b	54.12±4.22 ^a	66.84±4.21 ^b	85.54±6.33 ^a	1.55±0.64 ^a
440	150.52±4.38 ^c	55.38±5.08 ^a	78.26±3.69 ^b	84.39±6.14 ^a	1.72±0.69 ^a

Ui/Ut: Uninfected and untreated; I/Ut: Infected and untreated. ^{a,b,c}Values are mean±SEM of four trials. Values on the same column with the same letters are not significantly different at $P>0.05$.

3.9. Effect of treatment on serum bilirubin levels

From the Table 5 below, it appears that infection resulted in an increase of total and direct bilirubin as compared to neutral control. Administration of different doses of *L. abyssinica* and that of the mixture lead to a decrease in total and direct bilirubin compared to the negative control, while treatment with *V. paradoxa* at doses of 55 and 110 mg/kg, show a total and direct hyper-bilirubinemia compared to the controls. However, at doses 220 and 440 mg/kg, total and direct bilirubin has reduced to their normal value (that of the neutral control).

Table 5

Effect of treatment on total and direct bilirubin.

Doses of extract (mg/kg)	<i>V. paradoxa</i>		<i>L. abyssinica</i>		Mixture	
	Totale g/dL	Directe g/dL	Totale g/dL	Directe g/dL	Totale g/dL	Directe g/dL
Ui/Ut	1.38±0.03 ^a	0.15±0.07 ^a	1.38±0.03 ^a	0.15±0.007 ^a	1.38±0.03 ^a	0.15±0.007 ^a
I/Ut	1.72±0.25 ^b	0.25±0.03 ^b	1.72±0.25 ^b	0.25±0.03 ^b	1.72±0.25 ^b	0.25±0.03 ^b
55	4.22±0.45 ^c	1.28±0.27 ^b	1.29±0.12 ^a	0.74±0.30 ^f	1.57±0.17 ^{ab}	0.17±0.23 ^a
110	4.30±0.24 ^c	0.94±0.13 ^b	1.19±0.37 ^a	0.16±0.07 ^a	1.31±0.52 ^{ab}	0.17±0.05 ^a
220	1.14±0.24 ^{ab}	0.64±0.09 ^c	1.38±0.15 ^a	0.15±0.10 ^a	1.41±0.71 ^{ab}	0.14±0.07 ^a
440	1.55±0.30 ^{ab}	0.39±0.28 ^a	1.76±0.41 ^a	0.16±0.09 ^a	1.49±0.41 ^{ab}	0.14±0.09 ^a

Ui/Ut: Uninfected and untreated; I/Ut: Infected and untreated. ^{a,b,c}Values are mean±SEM of four trials. Values on the same column with the same letters are not significantly different at $P>0.05$.

4. Discussion

4.1. *In vivo anti-Salmonella effect*

The establishment of infection was clearly reflected by some changes in animals physiology including the excretion of watery stool, the presence of blood and mucus in the stool, the reduction of its activity and the exponential increase in the rate of *S. typhimurium* in the feces 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 defense mechanism of rats. The decrease of the bacterial load observed with treatment may be due to the combined action of the extract and immune system and given the fact that this decrease was also noted in the negative control group (infected and untreated). Animals treated at therapeutic healing dose (55 mg/kg) recovered in the same period as those treated with multiples of the therapeutic dose. This result suggests that this sample may have a higher activity *in vivo* due to their metabolism. The phytochemical screening revealed the presence of several classes of compounds in *V. paradoxa* leaf extract including phenols and polyphenols, flavonoids, alkaloids, tannins, saponins, cardiac glycosides and anthocyanins. Some of these secondary metabolites (flavonoids, alkaloids) have already shown several pharmacological properties including antibacterial properties[20].

The *in vitro* antioxidant activity reveals that *V. paradoxa* aqueous extract shows a weak DPPH radical scavenging activity with an IC₅₀ value of 320.03 µg/mL compared to the *L. abyssinica* extract of which exhibits an IC₅₀ value of 141.78 µg/mL.

Infection of rats with *S. typhimurium* has resulted in the modification of redox cell equilibrium stature. The administration of the different extracts over a period of 16 d does not only protect animals from oxidative stress but also scavenge free radical formed by infection.

The increases in the levels of total and LDL cholesterol treated animals with different doses of extracts may be due to the decrease in the activity of cholesterol 7α-hydroxylase, enzyme which catalyses the conversion of cholesterol into bile acids, which is the major route of elimination[21] due to the combined effects of infection and treatment. The increase in LDL-C may be the consequence of an increase in the activity of hepatic lipase and in turn to increase the degradation rate of HDL-C. Therefore, *S. typhimurium* infection is not a risk factor of cardiovascular disease since the arteriosclerosis index was not affected. Indeed, according to Schaffer and Menche[21], excess of “bad” cholesterol (LDL) and the lack of “good” cholesterol (HDL) are major risk factors for cardiovascular diseases. This hypothesis is supported by the rate of triglycerides which

have not undergone any significant changes during the different treatments. Indeed, triglycerides are carried in the bloodstream by chylomicron (cm)[22]. They are hydrolysed in circulation and their fatty acids are transported in the peripheral cells, leaving some particles which are harbingers of cardiovascular diseases[23]. Higher triglyceride levels measured in tissue of a fasting specimen indicate a lack of clearance or overproduction, which could increase the risk of developing cardiovascular disease[22].

4.2. *Protective effect of the extracts against the stress induced by infection*

Many environmental insults are known to cause oxidative stress, as indicated by LPO and H₂O₂ accumulation in cells[6,24]. Free radicals react with lipids and cause peroxidative changes, resulting in enhanced H₂O₂-generation and LPO[25], which eventually also, might negatively affect the activity of antioxidant enzymes in infected rats. Increased levels of MDA in the heart and kidneys in infected and not treated animals with the different extracts as compared to treated rats are due to a hyperoxidation of the tissues under the effect of infection. Infection could have resulted in a significant change in the cellular redox status in favor of prooxidants[2,26]. The significant decrease in total cholesterol and LDL compared to the negative control observed at dose level 55, 110 and 220 mg/kg (*V. paradoxa*) and 220 and 440 mg/kg (*L. abyssinica* and mixture) might be due to the presence of flavonoids in these extracts as revealed by phytochemical analysis[20]. In fact, flavonoids are chemoprotectors that fight against the harmful effect of free radicals, resulting in the maintenance of normal cytosolic MDA in kidney and heart of rats treated with the extracts and ascorbic acid (standard).

The results of this study showed that infection led to an imbalance of cytosolic redox status in favor of pro-oxidants, putting heart and liver cells in a state of oxidative stress, marked by the increase GSH levels, cardiac NO, and liver SOD activity, followed by a decrease in the activity of liver and kidney CAT in infected-untreated animals. But administration of different doses of extracts to infected animals has maintained the cellular antioxidant defence systems (GSH, SOD and CAT) to their normal level. Fridovich[27], reported that SOD played a key role in the detoxification of superoxide radical, thereby protecting cells from damage induced by free radicals. The observed decrease in SOD activity following *S. typhimurium* infection and treatment with different doses of plant extract might be due to the oxidation of CAT and GSH-Px enzymes. Farombi *et al.*[24], suggested that superoxide radicals by themselves, or after their formation to H₂O₂, caused oxidation of CAT and GSH-Px enzymes and thus decreased SOD activity.

Reduced GSH is an enzymatic biological antioxidant mostly present in the liver. It protects cellular proteins against

reactive oxygen species generated from exposure to pro-oxidant[28]. Decreased level of GSH is associated with increased LPO which is also confirmed in this study. The decrease in GSH levels in animals treated with different extracts might be due to the free radical's neutralizing activities of these extracts. They could have reactivated the hepatic GSH reductase which is reflected by decreasing the level of LPO. Our observations corroborated the report of Ebokaiwe *et al*[6]. This activity might be due to the presence of flavonoids, tannins, anthraquinones and phenolic compounds in these extracts whose free radical scavenger effects have already been shown.

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 LPO[29]. These results corroborate those of Yamaguchi *et al*. [30], which suggest that bilirubin serves as a physiological antioxidant in ischemia–infusion *in vivo*.

Literature has documented free radical generation during infectious diseases. The level of the markers of oxidative stress observed in negative control rats substantiates the possibility of extensive generation of free radicals during typhoid fever. The result of the present study shows that the extract of *V. paradoxa* is more active on *S. typhimurium* infected rat than that on *L. abyssinica*, where antioxidant activity is in contrary higher than that on *V. paradoxa*. These plant extracts have positive synergetic effect and can therefore be recommended for the treatment of typhoid fever and for the restoration of cell redox equilibrium during the bacterial infection.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Free radicals are paramagnetic molecular species which

increase in the body when the body's antioxidant system weakens. An oxidative stress is caused when the free radicals exceed the homeostasis level, leading to diseases. Oxidative stress is initiated by ROS such as hydrogen peroxide and superoxide anions, that react with the living tissues and cause harmful effects thereby causing diseases. Therefore, the efforts for the development of alternative antioxidants of natural origin have been reinforced.

Research frontiers

The objective of this study was to contribute to the development of alternative medicine through the study of the therapeutic properties (antibacterial and antioxidant) of *V. paradoxa* and *L. abyssinica* extracts *in vivo*.

Related reports

The level of the markers of oxidative stress, observed in infected rats, substantiates the possibility of extensive generation of free radicals during typhoid fever. The result of the present study shows that the extract of *V. paradoxa* is more active on *S. typhimurium* infected rat than *L. abyssinica* in which antioxidant activity is on contrary higher than that of *V. paradoxa*.

Innovations & breakthroughs

The present results demonstrate that aqueous extract of these plants have positive synergetic effect *in vivo*. Moreover, the ability of this plant to prevent the process of initiation and progression of free radical and infectious diseases may be attributed to the observed result. This plant extract can therefore be recommended for the treatment of typhoid fever and for the prevention of cell oxidation bacterial infection.

Applications

Antioxidant assays *in vivo* demonstrated that administration of these extracts to *S. typhimurium* infected rat positively and significantly affect the activities of enzymes and non–enzymes antioxidant. These results suggested that there were potent antibacterial and antioxidant activities which could be utilized as new natural antioxidant in antimicrobial therapeutics.

Peer review

By doing this work these authors have contributed to the development of alternatives drug from natural origin with positive synergetic effect and can therefore be recommended for the treatment of typhoid fever and for the restoration of cell redox equilibrium during bacterial infection.

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