International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2017.332.339

Review Article
Evaluation of Antibacterial and Antioxidant Properties of *Urtica urens* Extract Tested by Experimental Animals

[1]Taha Barkaoui, [1]Raoudha Kacem, [1]Fatma Guesmi, [2]Ahlem Blell and [1]Ahmed Landoulsi

[1]Laboratory of Biochemistry and Molecular Biology, Faculty of Science of Bizerta, Bizerta, Tunisia
[2]Pathological Anatomy Service, Regional Hospital of Menzel Bourguiba, Republic of Tunisia

Abstract

Background and Objective: Many plant extract have been reported to have an antimicrobial and antioxidative activities, for instance, *Salmonella typhimurium*, recognized as the main causes of food contaminations and may induce various human infections. In addition, hydrogen peroxide (H_2O_2) induced reactive oxygen species and the absence of their scavenge systems in cells leads to oxidative stress. The present study is focused on an essay to determine the antimicrobial and antioxidant properties of aqueous extract of *Urtica urens.* **Materials and Methods:** The antibacterial activity was tested in albino rats as a model using *S. typhimurium* infection. Mice were initially infected by *S. typhimurium* and then treated with *Urtica urens*-extract. Oxidative stress was induced in male Wistar rats by a single intraperitoneal injection of 1 mM of H_2O_2. **Results:** The extract (3 mg kg^{-1} b.wt.) treated animals was found to have significant effects on mortality and the numbers of viable *S. typhimurium* recovered from feces. The extract was fed to albino rats, followed by H_2O_2. Biochemical evaluation of the treatment has been tested at different enzymatic levels, such as glutathione (GSH), superoxide dismutase (SOD) and lipid peroxidation (MDA). The antioxidant assay showed a significant decrease of the MDA level and increase in the GSH and SOD. Although, clinical signs and histological damage were rarely observed in the treated mice, the controls showed a signs of lethargy and histological damage in the liver, spleen and intestine. **Conclusions:** *Urtica urens*-extract has the potential to provide an effective treatment for salmonellosis and oxidative stress.

Key words: *Urtica urens*-extract, antibacterial and antioxidant activities, lipid peroxidation, SOD, GSH

Received: January 31, 2016 Accepted: November 04, 2016 Published: March 15, 2017

Citation: Taha Barkaoui, Raoudha Kacem, Fatma Guesmi, Ahlem Blell and Ahmed Landoulsi, 2017. Evaluation of antibacterial and antioxidant properties of *Urtica urens* extract tested by experimental animals. Int. J. Pharmacol., 13: 332-339.

Corresponding Author: Taha Barkaoui, Laboratory of Biochemistry and Molecular Biology, Faculty of Science of Bizerta, 7021 Zarzouna, Tunisia
Tel: +21697736328

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Primary civilizations have used medicinal plants as the ultimate source of therapeutic aids[1]. In spite of the huge synthetic products into modern medicine, almost half of them are directly obtained from plants, many plants exhibit a unique complex combination of secondary metabolites which give an effective effect in therapy process for instance, caffeoylmalic acid, organic acid, chlorogenic acid, flavonoids, coumarins, steroids and scopoletin[2]. Moreover, aerial parts of plants are rich in inorganic minerals and vitamins etc. Which have significant antioxidant and antibacterial properties[3,4]. Many modern drugs which have contributed well in medical interventions were based or extracted from medicinal plants. Adequate drugs include the curare alkaloids, penicillin and other antibiotics, cortisone, reserpine, podophyllotoxin and other therapeutic agents[5].

Urtica urens (UU), which belongs to the family of Urticaceae and commonly known as nettle apple has been used extensively as a traditional medicine in many countries[6] for the treatment of anemia, rheumatism and arthritis, eczema, asthma, urinary gravel, stomach complaints, skin infections and as an anti-haemorrhagic[7].

Furthermore, *Urtica urens* is reported by an antioxidant and antibacterial effects[8,9]. The constituents of UU include caffeoylmalic acid, flavonoids, chlorogenic acid, gallic acid and caffeic acid which are well known for their therapeutic properties[2,10]. The main medicinal uses of nettles historically were internally as a tonic and highly nutrient food[11]. However, till the date, no studies regarding the antimicrobial and antioxidant activity of *Urtica urens* have been conducted. Therefore, the objective of the present study was to determine the protective effect of feeding the aqueous extract of *U. urens* to albino rats of the Wistar strain against *S. typhimurium* and the toxic effects of H_2O_2 by biochemical and histopathological methods.

MATERIALS AND METHODS

Plant material: *Urtica urens* is a perennial plant with stinging hairs belonging to the family Urticaceae, under the division Spermatophyta, subdivision of Angiospermae, class Dicotyledonae, group Apetalae, order Urticales. *Urtica urens* were collected from the Bizerta region of Tunisia on April, 2014 (South Mediterrranean). The botanical identification of *U. urens* was carried out by Professor Ben Nasri-Ayachi, Sciences Faculty of Tunisia.

Preparation of crude plant extracts: For aqueous extraction, 10 g of air-dried powder was boiled on slow heat in distilled water for 30 min. The extract then filtered using Whatman filter paper No. 1 and centrifuged at 5000×g for 10 min as discussed by Folcara *et al.*[12]. The extract supernatant was collected each 30 min and concentrated to a final volume equal to one fourth of the original one. The final solution was used to perform antibacterial and antioxidant activities. Finally, the supernatant was recovered and stored at -4°C until it is used.

Determination of *in vivo* antibacterial activity

Animal: Eighteen male Wistar rats (50-70 g) aged between 8 and 10 weeks mice were purchased from the Pasteur Institute (Tunisia) to perform all *in vivo* experiments. They were kept in a temperature-controlled room under a 12 h light 12 h dark cycle. Animals had free access to commercial solid food and water *ad libitum* and were acclimatized for at least 1 week prior to beginning the experiments. All mice experiments in this study were approved by the Bizerte University Animal Ethics Committee in accordance with the guidelines of the Tunisia Council on Animal Care.

Preparation of bacteria: *Salmonella typhimurium* (ATCC 14028) was used in this study. This strain was purchased from Institute Pasteur Tunis, stored at -80°C in glycerol stocks and used as required during different experiments.

***In vivo* assay using mice:** Mice were divided into the following groups: Control (CON), *Salmonella*-infected (SI) and *Salmonella*-infected+UU-extract (SIUU). Each group contained six mice. The growth inhibition of the test organisms in mice was then determined by monitoring *S. typhimurium* in the feces of the mice. Briefly, *S. typhimurium* was grown overnight in Luria–Bertani broth (Difco), centrifuged, washed in phosphate-buffered saline (PBS) and then diluted into 20% sucrose solution to achieve a final concentration of 1×10^5 CFU mL^{-1}. The SI and SIUU groups were then inoculated using gavage needle orally with 0.1 mL of already prepared bacterial suspension. Each day, 1 h after infection, 2 mL of UU aqueous extract were orally administered to all animals of the SIUU group (using gavage needle), whereas CON and SI animals were not. Fecal samples were then collected at 0, 1, 2, 3, 4, 5, 6 and 7 days after the bacterial suspensions were administered and the numbers of the bacteria per gram of feces were determined. Aliquots (100 µL) of fecal suspensions were serially diluted in PBS and then

plated on duplicate *Salmonella-Shigella* agar plates (Difco), which were subsequently incubated overnight at 37°C. Typical colonies were counted using the method of Lee *et al.*[13] on plates that contained between 30 and 300 colonies, after which confirmation of *S. typhimurium* was performed by a PCR assay using a previously described method[14]. At day 4 post-infection, the mice were sacrificed and tissue specimens of the liver, spleen and intestine organs were transferred to 10% buffered neutral formalin for histopathologic examinations and then processed using standard procedures. Sections of paraffin-embedded tissues were then stained with hematoxylin and eosin.

Determination of *in vivo* antioxidant activity

Experimental procedure: Hydrogen peroxide (H_2O_2) is a selectively toxic chemical agent. The H_2O_2 induced Reactive Oxygen Species (ROS) and/or a decrease the antioxidant defense mechanisms[15]. The ROS include free radicals. However, the increase in ROS and free radicals secretion was revealed to be an important cause among different biochemical manifestations in various diseases[16].

Stress was induced in male Wistar rats (50-70 g) by a single intraperitoneal injection according to Donnini *et al.*[17] of hydrogen peroxide (H_2O_2) at a dose of 1 mmol L^{-1} in 0.5 mL PBS. The animals were grouped into three groups containing six animals in each group. The first group served as control, the second group was administered H_2O_2 by intraperitoneal injection (negative control). The animals of the 2nd and 3rd groups were given dose of H_2O_2 at 1 mmol L^{-1} until the 14th day and the 3rd group was administered the aqueous extract of UU via oral route at 3-5 mg kg^{-1} b.wt., for 14 days. The dose was selected on the basis of the LD_{50} at the equivalent of up to 2 g dried drug kg^{-1} b.wt.[3]. Some of rats in first group were treated with physiological saline, daily for 14 days. They were housed at University Animal House in standard conditions and fed with standard diet with water *ad libitum*. At the end of experimental period, animals were sacrificed and the liver, spleen and small intestine were isolated to prepare homogenate.

Markers of oxidative stress: Animals were sacrificed and tissue was collected and then washed with ice-cold saline, weighed and minced, 10% homogenate was prepared in 0.15 M ice-cold KCl for TBARS (thiobarbituric acid-reactive substances), a marker for lipid peroxidation was estimated with the method of Ohkawa *et al.*[18] and protein was determined according to the method reported by Lowry *et al.*[19]. Measurements of glutathione were performed according to the method of Ellman[20] and superoxide dismutase concentration was determined according to the method of Marklund[21], using a teflon tissue homogenizer.

Tissue processing: Liver, spleen and intestine were flushed with chilled 1.15% (w/v) KCl solution. A 10% (w/v) homogenate was prepared in 50 mM phosphate buffer, pH 7.4 and centrifuged at 8000×g for 15 min at 4°C. Experiment were carried out according to the method described by Sen *et al.*[22]. The supernatant so obtained were used for the estimation of lipid peroxidation (MDA), glutathione (GSH) and superoxide dismutase (SOD). The protein content was determined by the method of Lowry *et al.*[19], using bovine serum albumin as the standard.

Statistical analysis: The results are expressed as Mean±SD of at least three sets of triplicate determinations for each data point. One-way ANOVA, Tukey and Dunnett tests were applied for analyzing the significance of difference between and among different groups.

RESULTS

***In vivo* antibacterial activity:** The *in vivo* antibacterial activity of UU-extract was examined using a mouse *S. typhimurium* infection model. Briefly, mice were infected with 1×10^5 CFU of *S. typhimurium* SI, 1 h late.

The UU-extract was orally administered to the mice. Table 1 shows that treatment with the extract of UU was found to have marked effects on mortality and on the number of viable *S. typhimurium* recovered from feces. At day 1 post-infection, 10 mice in the SI and SIUU group did not shed viable *S. typhimurium* in feces, whereas the feces of mice in the SI group being found to contain bacteria at a concentration of 1×10^2 to 2×10^3 CFU g^{-1} and feces of mice in the SIUU group being found to contain bacteria at a concentration of 0-4.3×10^3 CFU g^{-1}. In addition, at day 6 post-injection, one of the mice in the SIUU group had died, while all six mice in the SI group had succumbed.

Organ histopathologic changes: *Salmonella typhimurium* infected mice that did not receive the UU-extract were showed signs of histological damage in the liver, spleen and intestine. The central veins of the liver showed congestion with focal necrotic emboli-like materials. In spleen, an extensive hemorrhagic necrosis was detected in the red pulp

Table 1: Effects of treatment with UU-extract on fecal shedding of *S. typhimurium* (CFU g^{-1}) by mice

	Day of post-feeding						
Groups	0	1	2	3	4	5	6	7
SI_1	0	1.8×10^3	8.9×10^6	2.5×10^7	3.7×10^7	4.7×10^7	Death	Death
SI_2	0	2.4×10^3	1.5×10^4	1.6×10^6	2.6×10^5	1.2×10^6	8.6×10^6	Death
SI_3	0	1.2×10^2	4.8×10^5	2.6×10^6	3.6×10^6	1.9×10^7	Death	Death
SI_4	0	1.3×10^3	6.4×10^5	3.2×10^7	Death	Death	Death	Death
SI_5	0	1.38×10^3	1.9×10^4	5.8×10^6	3.26×10^7	Death	Death	Death
SI_6	0	1.7×10^3	5.2×10^4	1×10^4	1.8×10^5	2.8×10^5	3.2×10^6	Death
$SIUU_1$	0	0	0	2.6×10^2	6×10^5	3×10^3	7×10^2	1.7×10^2
$SIUU_2$	0	1.4×10^2	5.2×10^3	9.8×10^3	2.4×10^3	2.8×10^2	1×10^2	0
$SIUU_3$	0	0	2×10^3	8×10^3	3×10^3	6×10^2	3×10^2	2×10^2
$SIUU_4$	0	1×10^3	2.3×10^3	5.3×10^3	4.6×10^4	2.3×10^3	9.4×10^2	1.4×10^2
$SIUU_5$	0	4.3×10^3	2.9×10^4	1.3×10^6	6.5×10^6	2.3×10^7	1.8×10^8	Death
$SIUU_6$	0	0	3.2×10^3	1×10^4	1.9×10^4	1.4×10^3	3.2×10^3	1.2×10^2

SI: *Salmonella*-infected, SIUU: *Salmonella*-infected+UU

Table 2: Level of MDA in liver, spleen and small intestine of control and experimental animals in each group

	TBARS (nmol g^{-1} tissue)		
Parameters	Liver	Spleen	Small intestine
Group I: Control rats	51.23±4.9	8.34±0.91	17.19±2.68
Group II: Rats intoxicate with H_2O_2	123.32±11.5***	17.31±0.80***	28.76±4.93***
Group III: Rats intoxicate then treated with aqueous extract(UU)	55.45±5.51	10.32±2.30	14.42±2.30

Values are expressed as Mean±SD (n = 6). ***Significantly different from control at p<0.001

Table 3: Level of GSH in liver, spleen and small intestine of control and experimental animals in each group

	GSH (μmol g^{-1} tissue)		
Parameters	Liver	Spleen	Small intestine
Group I: Control rats	42.66±2.86	21.63±2.32	43.23±2.65
Group II: Rats intoxicate with H_2O_2	12.22±1.46***	7.32±0.72***	16.74±1.14***
Group III: Rats intoxicate then treated with aqueous extract (UU)	31.99±4.42	18.23±2.84	40.83±2.44

Values are expressed as Mean±SD (n = 6), ***Significantly different from control at p<0.001

with multiple apoptotic bodies in the white pulp. In addition, destruction and atrophy with ischemic necrosis and edematous changes with polymorphonuclear leukocyte infiltration within the mucosal layers of the small intestine were evident. In contrast, clinical signs and histological damage were rarely observed in *S. typhimurium*-infected mice fed the extract of UU-extract (Fig. 1).

***In vivo* antioxidant activity:** Effect of UU-extract on lipid peroxidation status: Table 2 represents the levels of lipid peroxidation (TBARS) in the liver, spleen and small intestine of control and experimental animals. A significant increase in the levels of TBARS was observed in the hydrogen peroxide (H_2O_2) alone treated animals (Group II) when compared with control animals (Group I). This was significantly reversed to near normal levels in *Urtica urens* (3 mg kg^{-1} b.wt.) treated animals (Group III). The UU-extract treated animals (Group III) did not show any significant variations when compared to control (Group I) animals. According to these observations UU-extract may induce the protection against the H_2O_2 induced oxidative stress by reducing the lipid peroxidation.

Effect of UU-extract on GSH status: Table 3 shows the level of non-enzymic antioxidant (GSH) in liver, spleen and small intestine of control as well as treated animals. The GSH status was found to be significantly lowered in H_2O_2 alone treated animals (Group II) when compared with control animals (Group I). The alterations of GSH-levels were reverted to nearly control values on the administration of UU-extract treated animals (groups III) when compared with (group I) animals. Animals intoxicate then treated with UU-extract (Group III) did not show any significant variations when compared to control (Group I) animals.

Effect of UU-extract on superoxide dismutase (SOD): Rats intoxicate with H_2O_2 had significantly low levels of SOD activity compared to the control rats. However, Animals intoxicate then treated with UU-extract (Group III) to show a

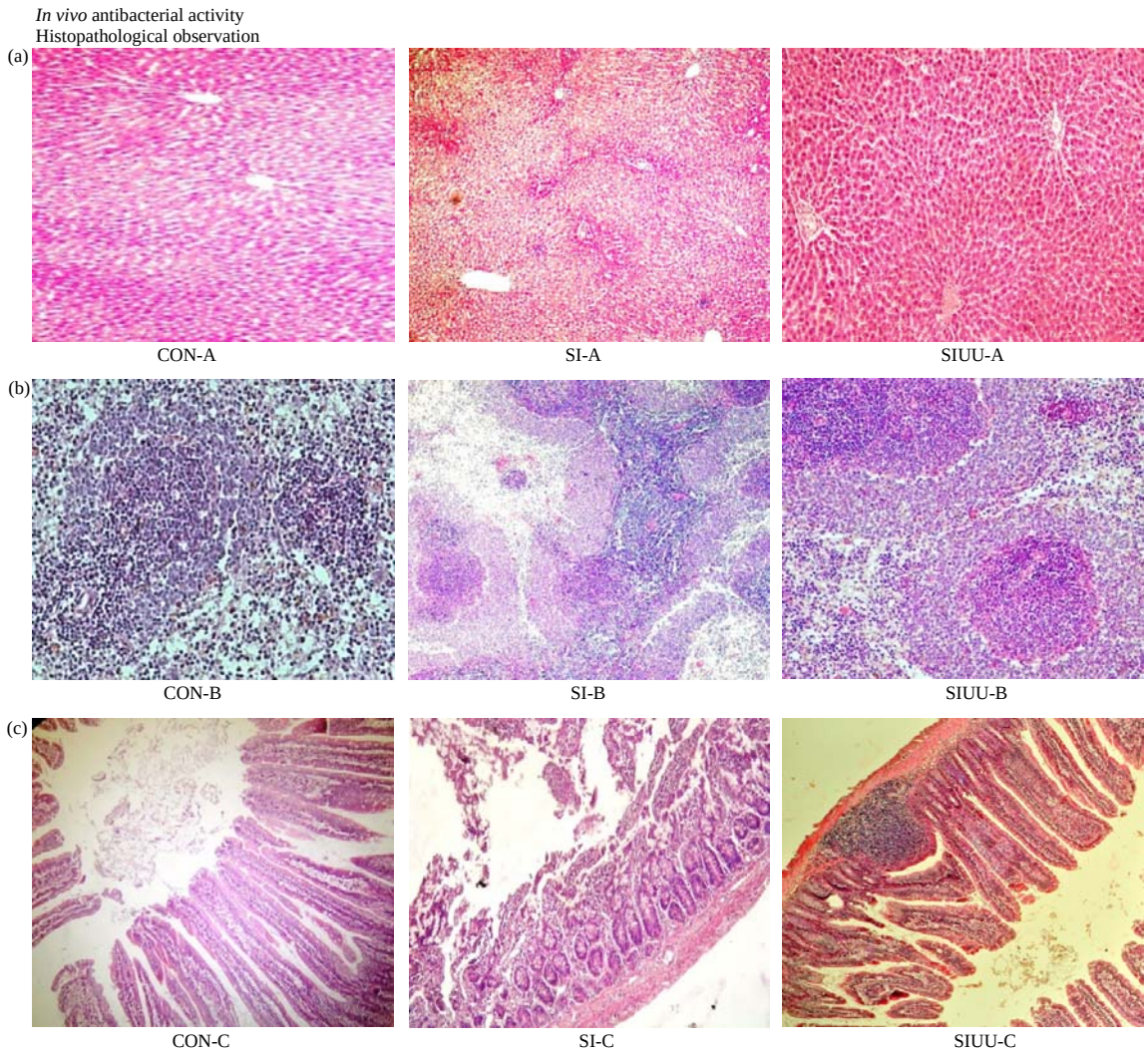

Fig. 1(a-c): Histopathological changes in organs in CON, SI and SIUU. (a) Liver (×200), (b) Spleen (×200) and (c) Small intestine (×200). CON-A control: Normal hepatocytes showing normal architecture with portal tried, showing portal veins, hepatic artery and vein. SI-A congestion and edematous changes within the central and portal veins of the liver and severe hemorrhagic necrosis was also observed within the red pulp of the (SI-B). In addition, destruction and atrophy with ischemic necrosis within the mucous layers of the small intestines where observed (SI-C). *Urtica urens*-fed mice (test group) infected with *S. typhimurium*. Histological damages in the above organs were rarely observed in these mice (SIUU)

significant ($p<0.001$) increase in SOD levels when compared with the control group (Table 4).

Organ histopathologic change: Group II animals were lethargic and showed signs of histological damage in the liver, spleen and small intestine. The central and portal veins of the liver showed congestion with focal necrotic emboli-like materials. The histological photomicrographs of the spleen sections are shown in Fig. 2. The congestion of the spleen tissue was showed in the H_2O_2-treated group, while no severe damages and lymph nodule proliferation of spleen tissue were observed in group III animals. In addition, destruction, atrophy and edematous changes with polymorphonuclear leukocyte infiltration within the mucous layers of the small intestines were observed. Conversely, clinical signs and histological damage were rarely observed in H_2O_2 intoxicated-mice fed with the UU-extract (Fig. 2).

DISCUSSION

In the present study, *Urtica urens*-extract was screened for antibiotic activity against several pathogenic *Salmonella*

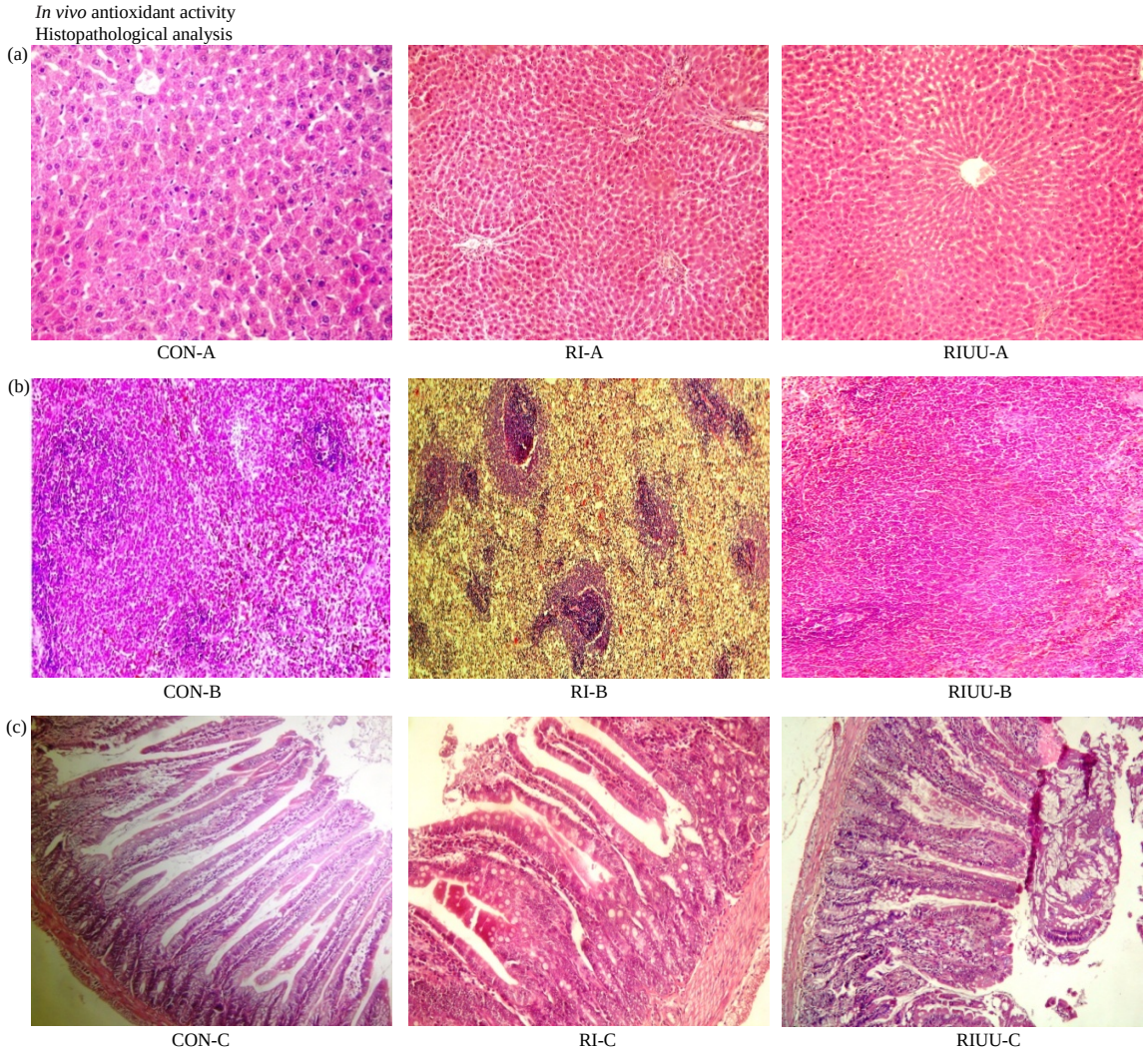

Fig. 2(a-c): Histopathological changes in organs in CON, RI and RIUU. (a) Liver, (b) Spleen and (c) Small intestine, CON: Control rat, RI: Rats intoxicate with H_2O_2 alone (negative control), RIUU: Rats intoxicate with H_2O_2 then treated with aqueous extract of *Urtica urens*, (H and E X200)

Table 4: Level of SOD in liver, spleen and small intestine of control and experimental animals in each group

	SOD (U mg^{-1} protein)		
Parameters	Liver	Spleen	Small intestine
Group I: Control rats	17.37±2.27	8.22±0.63	15.95±0.14
Group II: Rats intoxicate with H_2O_2	4.88±0.71***	2.17±0.15***	7.60±1.16***
Group III: Rats intoxicate then treated with aqueous extract (UU)	13.75±2.26	7.00±0.31	14.89±0.36

Values are expressed as Mean±SD (n = 6). ***Significantly different from control at p<0.001

serotypes. The *in vivo* antibacterial assay revealed that the extract showed the effective inhibition of *S. typhimurium* growth and significantly reduced mice mortality (Table 1). Furthermore, clinical infection signs and histological damage were rarely observed in the SIUU-group (Fig. 1), whereas infected mice SI showed severe clinical signs and histological damage in the considered organs. This is the first report to describe the antibacterial activity of UU-extract against *S. typhimurium.* Based on this promising *in vivo* assay results it is clearly proved that UU-extract can be considered like a novel antimicrobial treatment for salmonellosis. The UU-extract has been reported only to show the antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*

delivery as reported by Leven *et al.*[23]. The antibacterial activity of UU-extract may be indicative of the presence of some metabolic toxins or broad-spectrum antibiotics. Several metabolites from herb species, such as, alkaloids, tannins, saponins and sterols have been previously associated with antimicrobial activity reported by Taguri *et al.*[24]. The major chemical constituents of *Urtica urens* are flavonoids, caffeoyl-esters, caffeic acid, scopoletin (cumarin), sitosterol (-3-O-glucoside), polysaccharides, fatty acids (e.g., 13-hydroxy-octadecadienoic acid), minerals (herba: up to 20% leaves: 1-5%) as discussed by Doukkali *et al.*[25] And the aerial parts are rich in minerals and vitamins and *Urtica urens* was reported to show anti-food-borne pathogens.

Furthermore, antioxidant activity is one of the most intensively studied subjects in aqueous plant extract. In this study, the therapeutic effects of UU-extract were studied by examining the prevention of hydrogen peroxide induced stress in rats. The H_2O_2 is one of the most widely used toxicant for experimental induction of liver, spleen and intestine in laboratory animals.

Malondialdehyde is generated from the degradation of polyunsaturated lipids by ROS. It is one of the most frequently used indicators of lipid peroxidation[26], in this study we have demonstrated that elevated levels of MDA in H_2O_2-induced rats were reduced after the treatment with UU-extract (Table 2).

Glutathione is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, it is noteworthy to cite the study of Swaroop and Ramasarma[27]. In the present study, significantly low GSH levels were observed in rats intoxicated with H_2O_2 as compared to the control. While UU-extract treatment showed significant increase above normal level. Thus, we noted that UU-extract may offer better antioxidant effect by scavenging free radicals and restoring the imbalance between oxidant/antioxidant homeostasis developed during stress condition (Table 3).

Antioxidant enzymes such as SOD have been shown vital to eliminate ROS. The SOD is the most important antioxidant enzyme that inhibit free radical formation and is usually used as biomarker to indicate ROS production[28]. The SOD is one of the important enzymes that scavenges superoxide radical ($O_2^{\cdot-}$) to H_2O_2 and molecular oxygen[29]. Table 4 depicts the levels of SOD activity in liver, spleen and intestine tissues of the intoxicate rats with H_2O_2 followed by UU-extract treatment. The treatment of rats by 1 mmol L^{-1} of H_2O_2 until the 14th day reduces significantly the levels of SOD by (71.9, 73.61 and 52.36%), respectively. The increase in SOD activity post-operatively was indicative of restoration of antioxidant defense system in the controls groups. This result was in agreement with that reported by Zheng *et al.*[29]. Histopathological studies carried out for the liver, spleen and small intestine of control group, H_2O_2 treated and UU-extract treated results are given in Fig. 2. The massive generation of free radical in the H_2O_2-induced tissues damages provokes a sharp increase of lipid peroxidation. On the other hand, it reduces significantly the of GSH and SOD levels in liver, spleen and intestine, respectively.

The results of this study were supported by similar observation in the others researchers[30,31] that H_2O_2 was able to induce oxidative stress in these tissues. In case of H_2O_2-treated rats, strong modification in organ architecture and areas of hemorrhage and necrosis were seen. However, in the case of group III, the liver, spleen and small intestine were shown to retain normal architecture with few areas of hemorrhage (Fig. 2).

In this study, the results showed that UU-extract treatment prevented H_2O_2-induced stress in rats by strengthening the antioxidant defense system. Therefore, these results demonstrated that the UU-extract has protective function against H_2O_2 toxicity in rat liver, spleen and small intestine. Similar results have been reported for some other ethnobotanical fruits and herbs, in agreement with the analysis of Kim *et al.*[32]. The results of the present study may have very important implications for the chemopreventive potentials antibacterial and antioxidant profiles of aqueous extract of *U. urens* as a traditional herbal medicine.

CONCLUSION

This study may suggest new treatments in the curative of salmonellosis and oxidative stress reveals the importance of scientific research on miscellaneous plants with various medicinal properties. Further studies are required to evaluate the possible interactions of *U. urens* with therapeutic drugs and/or other dietary components in order to clarify its possible use as traditional medicinal herb.

ACKNOWLEDGEMENT

We thank Prof. Ben Attia Msaddek from Science of live department of Faculty of Science of Bizerta, for scientific discussion and advice.

REFERENCES

1. Chrubasik, J.E., B.D. Roufogalis, H. Wagner and S.A. Chrubasik, 2007. A comprehensive review on the stinging nettle effect and efficacy profiles. Part II: Urticae radix. Phytomedicine, 14: 568-579.

2. Frank, B., I. Bohn and B. Uehleke, 1998. Urtica. In: Hager's Handbuch der Pharmazeutischen Praxis, Blaschek, W., R. Hansel, K. Keller, J. Reichling, H. Rimpler and G. Schneider (Eds.). Springer, New York, USA., pp: 710-736.
3. ESCOP., 2003. ESCOP Monographs. 2nd Edn., Thieme, Stuttgart, Germany, ISBN: 9781588902337, pp: 521-527.
4. Steenkamp, V., E. Mathivhaa, M.C. Gouwsb and C.E.J. van Rensburga, 2004. Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. J. Ethnopharmacol., 95: 353-357.
5. Andraws, R., P. Chawla and D.L. Brown, 2005. Cardiovascular effects of ephedra alkaloids: A comprehensive review. Progr. Cardiovasc. Dis., 47: 217-225.
6. Randall, C., 2003. Various Therapeutic Uses of Urtica. In: Urtica: Therapeutic and Nutritional Aspects of Stinging Nettles, Kavalali, G.M. (Ed.). Taylor & Francis, London, UK., pp: 40-46.
7. Arslan, S., G. Terzioglu, S. Elcil, H. Deligoz and A. Sen, 2014. Assessing of anti-inflammatory effect of small nettle (*Urtica urens*) increasing polarity extracts. J. Neuroimmunol., 275: 135-135.
8. Keles, O., T. Bakirel, S. Ak and A. Alpmar, 2001. The antibacterial activity of some plants used for medicinal purposes against pathogens of veterinary importance. Folia Vet., 1: 26-31.
9. Toldy, A., K. Stadler, M. Sasvari, J. Jakus and K.J. Jung *et al*., 2005. The effect of exercise and nettle supplementation on oxidative stress markers in the rat brain. Brain Res. Bull., 65: 487-493.
10. WHO., 2002. WHO Monographs of Selected Medicinal Plants. Vol. 2, World Health Organization, Geneva, pp: 125-135.
11. Barnes, J., L.A. Anderson and D.J. Philipson, 2002. Herbal Medicines: A Guide for Healthcare Professionals. 2nd Edn., Pharmaceutical Press, London, pp: 360-364.
12. Folcara, S.C., R.V. Casanovas and M. Wichtl, 1998. Medicinal Plants and Plant Drugs: For Infusion and Tisane: A Scientifically Based Handbook for Pharmacists and Physicians. OEMF International, Milano, Italy.
13. Lee, M.H., H.A. Kwon, D.Y. Kwon, H. Park and D.H. Sohn *et al*., 2006. Antibacterial activity of medicinal herb extracts against *Salmonella*. Int. J. Food Microbiol., 111: 270-275.
14. Alvarez, J., M. Sota, A.B. Vivanco, I. Perales, R. Cisterna, A. Rementeria and J. Garaizar, 2004. Development of a multiplex PCR technique for detection and epidemiological typing of *Salmonella* in human clinical samples. J. Clin. Microbiol., 42: 1734-1738.
15. Heyman, S.N., S. Rosen and C. Rosenberger, 2011. A role for oxidative stress. Contrib. Nephrol., 174: 138-148.
16. Whaley-Connell, A., P.A. McCullough and J.R. Sowers, 2011. The role of oxidative stress in the metabolic syndrome. Rev. Cardiovasc. Med., 12: 21-29.
17. Donnini, M., M. Luidetti and L. Diomede, 1990. Ambroxol reduces paraquat toxicity in the rat. Prog. Respir. Res., 25: 329-332.
18. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
19. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.
20. Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys., 82: 70-77.
21. Marklund, S.L., 1985. Pyrogallol Autooxidation. In: CRC Handbook of Methods for Oxygen Radical Research, Greenwald, R.A. (Ed.). CRC Press, Boca Raton, Florida, pp: 243-244.
22. Sen, A., B. Sahin, H.H. Agus, M. Bayav, H. Sevim and A. Semiz, 2007. Prevention of carbon tetrachloride-induced hepatotoxicity by *Urtica urens* in rats. J. Applied Biol. Sci., 1: 29-32.
23. Leven, M., D.A.V. Berghe, F. Marten, A. Vilientnick and E. Lommens, 1979. Screening of higher plants for biological activities. I. Antimicrobial activity. Planta Med., 36: 311-321.
24. Taguri, T., T. Tanaka and I. Kouno, 2004. Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. Biol. Pharm. Bull., 27: 1965-1969.
25. Doukkali, Z., H. Bouidida, A. Srifi, K. Taghzouti, Y. Cherrah and K. Alaoui, 2015. [Anxiolytic plants in Morocco: Ethnobotanical and ethno-pharmacological study]. Phytotherapie, 13: 306-313.
26. Scholz, R.W., K.S. Graham, E. Gumpricht and C.C. Reddy, 1989. Mechanism of interaction of Vitamin E and glutathione in the protection against membrane lipid peroxidation. Ann. N. Y. Acad. Sci., 570: 514-517.
27. Swaroop, A. and T. Ramasarma, 1985. Heat exposure and hypothyroid conditions decrease hydrogen peroxide generation in liver mitochondria. Biochem. J., 226: 403-408.
28. Queguineur, B., L. Goya, S. Ramos, M.A. Martin, R. Mateos and L. Bravo, 2012. Phloroglucinol: Antioxidant properties and effects on cellular oxidative markers in human HepG2 cell line. Food Chem. Toxicol., 50: 2886-2893.
29. Zheng, Y., Y. Liu, J. Ge, X. Wang, L. Liu, Z. Bu and P. Liu, 2010. Resveratrol protects human lens epithelial cells against H_2O_2-induced oxidative stress by increasing catalase, SOD-1 and HO-1 expression. Mol. Vis., 16: 1467-1474.
30. Aruoma, O.I., 1994. Nutrition and health aspects of free radicals and antioxidants. Food Chem. Toxicol., 32: 671-683.
31. Hsiao, G., M.Y. Shen, K.H. Lin, M.H. Lan and L.Y. Wu *et al*., 2003. Antioxidative and hepatoprotective effects of *Antrodia camphorata* extract. J. Agric. Food Chem., 51: 3302-3308.
32. Kim, K.S., S. Lee, Y.S. Lee, S.H. Yung, Y. Park, K.H. Shin and B.K. Kim, 2003. Anti-oxidant activities of the extracts from the herbs of *Artemisia apiacea*. J. Ethnopharmacol., 85: 69-72.