المؤتمر العلمي الدولي الاول للتقنيات الطبية والبيولوجية / للفترة 8-9 آذار 2017
Al-Kufa University Journal for Biology
Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Study of anti- inflammatory effect of *Tribulus terrestris* extract on bacterial infected wounds in mice

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Summary

The effect of water extract from *Tribulus terrestris* was studied as an anti-inflammatory agent on albino mice with *Staph aureus* infected wounds and investigating the toxicity of this substance. Two groups with ten mice in each one were used to conclude this part. The first one served as control that was wounded and infected by *Staphylococcus aureus* and left for normal healing , while the other group was treated with the extract after wounds were contaminated and infected with S*taphylococcus aureus.* The results showed an accelerated healing in wounds treated with *T. terrestris* compared with control. The cytotoxicity was performed on three groups of mice; every group included ten mice divided as: control group, the second group was orally administrated with 75mg/kg of the aqueous extract; the third group was administrated orally with 25mg/kg of the aqueous extract. The administration last for 10 days. After 10 days, mice were killed and put for autopsy histology slides were made from kidney and liver and compared with control group. No change was observed among studied slides which may indicate that the extract has no toxic effect on organs.

Key words: *Tribulus terrestris*, anti-inflammatory, cytotoxicity

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Introduction

The genus *Tribulus terrestris* (Zygophyllaceae) comprises with 20 species which grow as shrubs in subtropical areas around the world and only two species distributed in China, *T. terrestris* and *T. cistoides*. In traditional Chinese medicine, the fruit of *T. terrestris*, which is known as "Ci Ji Li", has been used against diverse diseases for a long time. The crude saponin fraction of the whole plant has been used as a convivial drug *Tribulus terrestris* (L.,) is also known as puncture vine or small caltrops 10 to 60 cm height, annual herb, with pinnate leaves and yellow flowers . Its carpel fruits are very distinguishing in nature and are known as "Chih-hsing" in China or "Goat head" in the USA. The plant can be found in arid climate regions around the world as in southern USA, Mexico, Spain , Bulgaria, India, and China (Mahammad *et al*., 2012).

The fruits and seeds are of immense importance in oriental medicine because they are used as an aphrodisiac, diuretic and anthelmintic, as well as to treat coughs and kidney failure *Tribulus terrestris* L. has reported to have antimicrobial, antihypertension, diuretic, antiacetylcholine, hemolytic activity , stimulate spermatogenesis, libido, antitumor activity and effects on cardiovascular system (Handa and Kau, 2006).

Taxonomy of the Plant

Class Dicotyledons
Sub class Polypetatae
Series Thalamiflorae
Order Geraniales
Family Zygophyllaceae

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Genus *Tribulus*
Species *terrestris*8

Phytochemistry:

Furostanol and spirostanol saponins, flavonoid glycosides, alkaloids and some amides have been reported to this Traditional medicine. The major constituents of this plants are steroidal saponins named terrestrosins A, B, C, D and E, desgalactotigonis, F-gitonis, desglucolanatigoneis, gitnin, which on hydrolysis yield diosgen ins, ecogenins and neotigogenin Four pairs of sapogenins, tigogenin and neotigogenin, gitogenin and neogitogenin, hecogenin and neohecogenin, and manogenin and neomanogenin, have been isolated through hydrolysis of the crude saponins of *T. terrestris* (Devi and Ramesh, 2008).

Inflammation is one of the oldest known diseases of mankind and affects a large population of the world. The search of screening and development of drugs for analgesic and anti-inflammatory is an everlasting problem. There is much hope of finding anti-inflammatory drugs from native plants, as these are still used in therapeutics despite the progress made in conventional chemistry and pharmacology for producing effective drugs (Lee and Surh, 2012).

The practice of plants, plant extracts or plant-derived pure chemicals to manage disease become a therapeutic modality, which has stood the test of time. As assumed by the World Health Organization (WHO), about three-quarters of the world population depends upon traditional remedies (mainly herbs) for the health care of its people. The traditional medicines also some time called as, herbal or natural medicine existed in one way or another in different cultures/civilizations, such as Egyptians, Western, Chinese, Kampo (Japan) and Greco-Arab or Unani/Tibb (South Asia) .

Material and Method

Plant material: Purchase from local market.

Method of extraction: A quantity of 50 g of the flower powder was mixed with 250 ml sterile double distilled water. The mixture was left in a shaker incubator at 50^{o}C for 24 hours, than filtered through a filter paper (Whatman No. 1). The filtrate was concentrated using rotary evaporator at 40^{o}C until dryness and the extract residue was weighted and kept until use (Adaikan *et al*., 2000).

Microorganism isolate: The following pathogenic microorganism was used in this study,Which was obtained from the Dept. of Biotechnology, College of Science, Al-Nahrain University : *Staph. aurous.*

Experimental animals: Female albino mice (22–29 g) were obtained from the animal house of Biotechnology Dept., College of Science, Al- Nahrain University.

Preparation of animals and experimental design: Thirty female albino mice (22–29 g) were divided into three groups of ten animals and they were treated orally with *Tribulus terrestris* extract at different doses for 10 days and divided into two groups of ten animals studies showed anti-inflammatory effects, **Histopathology** (Group A: Normal, Group B

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75mg/kg and group C 25mg/kg : *Tribulus terrestris* extract), **Anti – inflammation (**Group D: *Tribulus terrestris* extract treatment skin, Group E:Control).

At the end of the experiment, animals were sacrificed and collected by Kidney and liver was keep at 10% formalin. Histopathological Examination (Bancroft and Stevens, 1992).

Detection o f Saponins: The plant extract 5 ml was added to 3 ml of mercuric chloride solution ($HgCl_3$). The appearance of white precipitate indicates the presence of saponins and also the appearance of big foam for a long time as a result of stirring the aqueous solution of plant powder in test tube indicates to saponins existence (Su *et al*., 2012).

Detection of Alkaloids: Five ml of plant extract and 2 ml of marquis reagent were added in test tube. When shaking the tube, the appearance of gray precipitate is an indication of the presence of Alkaloid (Su *et al*., 2012).

Detection of Flavonoids: Ten ml of 50% ethyl alcohol were mixed within 10 ml of 50% potassium hydroxide solution. Then they were added to equal volume of plant extract. The appearance of yellow colour indicates the presence of Flavonoids (Su *et al*., 2012).

Detection of Terpenes and Steroid: One gram of plant extract was dissolved in 1-2 ml of chloroform and then a drop of acetic anhydride and drop of concentrated sulfuric acid were added. Then the appearance of brown color represents the presence of terpenes. After some time, if a dark blue color was appeared it indicates the presence of steroid (Su *et al*., 2012).

Result and discussion

Medical plants are defined as any plant or any part of it that participate in keeping health in healthy bodies and prevention of disease. There are so many evidence leads to the use of humans for the plant sources for medical purposes from decades ago before history. A variety of plants are reported to possess aphrodisiac potentials. For centuries, Arabs have made use of herbal drugs to improve sexual performance and increase libido. One of aphrodisiac plant used by Arabs was *T. terrestris* . It has played an important role in the folk medicine of many countries (Al-Ali *et al*., 2003).

Histopathology.

The histopathology study of animal with treated plant extract is compared with, control and photos illustrating difference is shown in figure (1), Histopathology of liver, and kidney sections of animal treated and control animals showed the normal tissue.

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Print ISSN: 2073-8854 & Online ISSN: 2311-6544

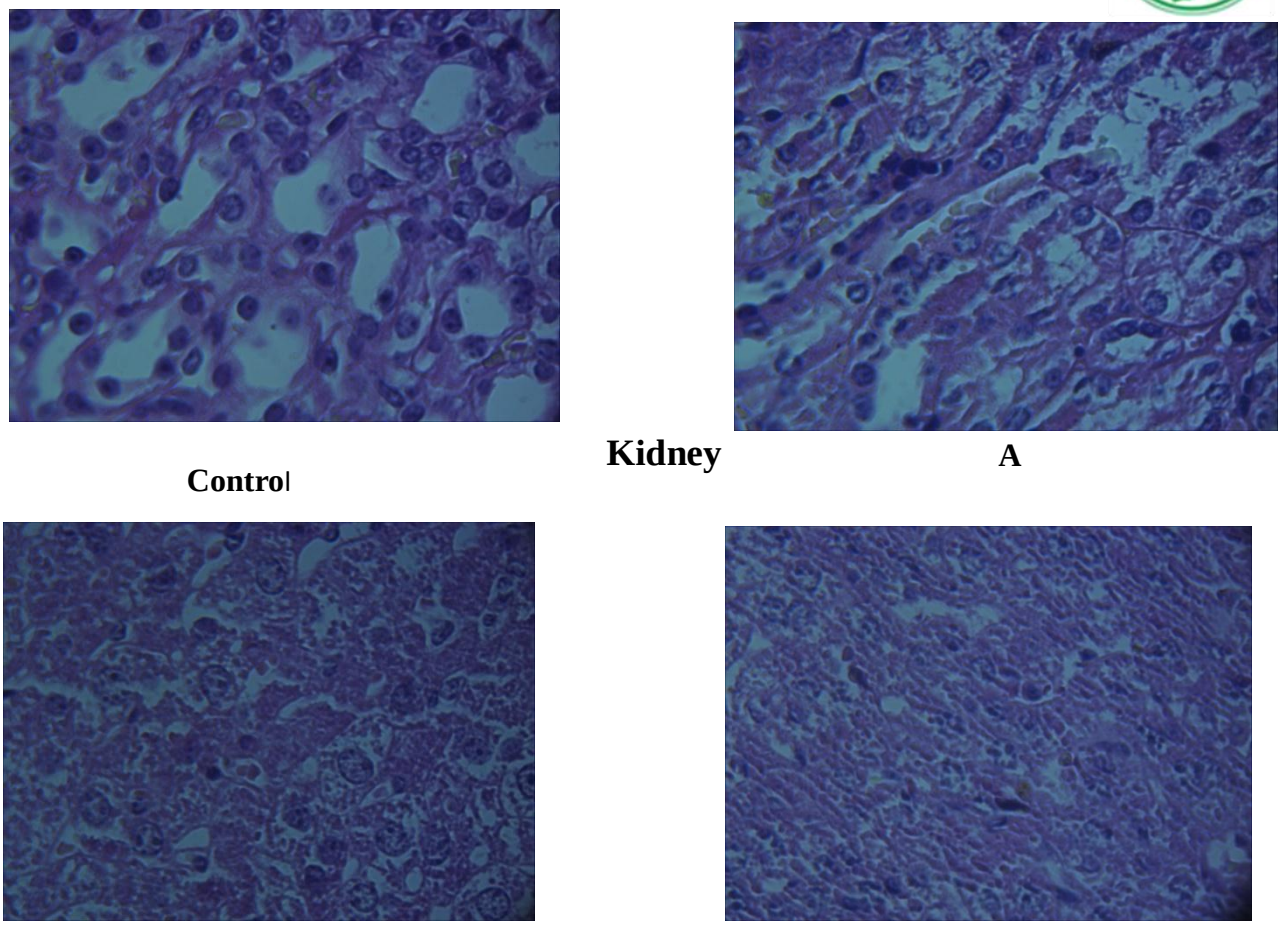

Figure. (1). Cross-section kidney and liver mouse administrated with *T. terrestris* extract . Shows control compere with (A) treated. (H&E) stain X400.

The figure (1) no change was observed among studied slides which may indicate that the extract has on toxic effect on organs

Anti-Inflammatory effect in animal models of *T. terrestris.*

Inflammation is defined as the reaction of vascularised living tissue to injury. The inflammatory response is closely intertwined with the process of repair. Inflammation serves to destroy, dilute or wall off the injurious agent, but in turn it sets into motion a series of events that as far as possible heal and reconstitute the damaged tissue. Repair begins during the early phases of inflammation but reaches completion usually after the injurious influence has been neutralized. Inflammation, however, if runs unchecked, leads to onset of vasomotor rhinorrhoea, rheumatoid arthritis, hypersensitivity reactions, fetal renal disease and atherosclerosis. These drugs have wide range of chemical nature and share, the common mechanism of action, pharmacological actions and adverse effect profiles. Although these drugs are effective in controlling signs of inflammation, numbers of adverse effects encountered are the biggest limitations to their use. Because of the side effect profile of NSAIDs, patients are inclined to choose the alternative system of treatment. Dashamula, a combination of roots of ten plants, is standard Ayurvedic remedy for inflammatory disease (BABURAO *et al*., 2009).

The anti - inflammation study of animal with treated plant extract is compared with, control and photos illustrating difference is shown in figure (2).

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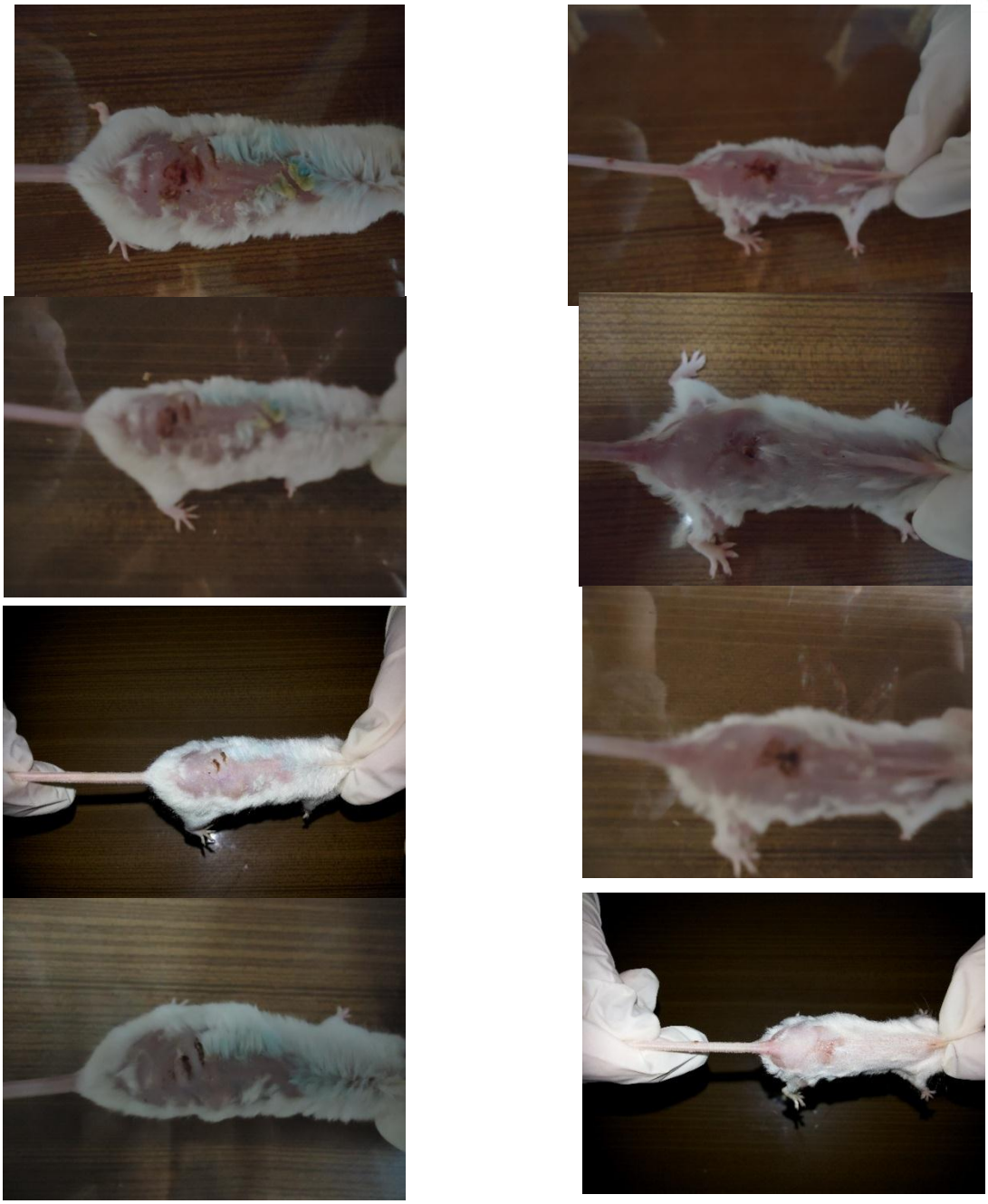

A **B**

Figure. (2). Anti-Inflammatory, treated with *T. terrestris* extract (A), and untreated control (B).

The figure (2) showed an accelerated healing in wounds of extract treated group compared with control.

The anti-inflammatory activity of *T. terrestris* flowers cultured in Europe and Asia has been evaluated and evidenced through the model of edema induction of the ear through croton oil and the model of edema induction of the paw through carrageenan. The angiogenic activity of the aqueous extract of *T. terrestris* cultured in England was also evidenced (Shraddh *et al.*, 2010).

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Detection of Some Active Compounds in *Tribulus trrestris* L. aqueous extract.

The results in table (1) show active compounds of aqueous extract of *Tribulus terrestris*.

Table. (1). Aqueous Active Compoundsin *T. terrestris* extract

No.	Active compounds	Aqueous extract of *T. terrestris*
1.	Tannins	–
2.	Saponins	+
3.	Alkaloids	+
4.	Flavonoids	+
5.	Terpenes	+
6.	Steroid	+

The symbol (+) refers to presence of compound and the symbol (−) refers to absence of compound.

Tribulus contains biologically active substances as steroids, saponins, flavonoids, alkaloids. Glycoconjugates are a class of complex molecules that are widely distributed in the plant kingdom and in some marine organisms. This class of compounds has a wide range of biological activates as anti-inflammatory, antimicrobial, antifungal, anticancer and other benefits. Among these compounds, steroidal and triterpenoid saponins have long been known as components of widely used herbal drugs and pharmaceutical preparation (Javed *et al.*, 2012).

The saponins are distinguished as steroidal saponins and triterpenoid. The steroidal saponins include the following chemicals: terrestrosin, diosin, gracillin, kikuba, PTN, nohecogenin, glucoside, tribulosi and F-gitonis (Lee.,2012).The other constituents are flavonoids which include the following substance: Kaempferol, kaempferol glycosides and quercertin. The other minor constituents are present like fatty acids (palmitic, stearic, oleic and linoleic) acids (Ka ul *et al.*, 2012).

Tribulus also contains small amounts of alkaloid, tannin, potassium salts, cinnamic amide, resine and sugar. It also presents fixed and essential oils, porphyrin, saprogenic ,25 species of flavonoids-glycosides and resin . Isolation of four furostanol saponins are known as: methylprotodioscin, protodioscin, methylprototribestin and prototribestin. The quality of this herb is dependent on the PTN content (Mona *et al*., 2014).

References

Al-Ali, M.; Wahbi, S.; Twaij, H. and Al-Badr, A. (2003). *Tribulus terrestris*: preliminary study of its diuretic and contractile effects and comparison with *Zea mays*. Journal of Ethnopharmacology 85: 257–260.

Adaikan, P. G.; Gauthaman, K.; and Prasad, R. (2000). Protractile pharmacological effects of *T. terrestris* extract on the rabbet corpus cavernosum. *Annals Academy of Medicine.* 29(1):22-26.

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Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Baburao,B.; Rajyalakshmi, G.; Venkatesham,A.; Kiran,G.; Shyamsunder,A.; and Gangarao,B.(2009). Anti- inflammatory and anti- microbial activities of methanolic extract of *Tribulus terrestris*. Int. J. Chem. Sci.: 7(3): 1867-1872.

Bancroft, J. D.; and Stevens, A.S.(1992). Theory and Practice of Histological Techniques. 2nd (ed.) Churchill Living Stone, Edinburgh, London.

Devi, DJ. and Ramesh, CU.(2008). Different chemo types of (*Tirbulus terrestris*): a herb used for improving physique and physical performance. Int J Green Pharmacy . 3: 158–161.

Handa, S. and Kaul, MK. (2006).Supplement to Cultivation and Utilization of Medicinal Plants, Jammu-Tawi: Regional Research Laboratory. 566.

Javed Akhtar Ansaria ; Mateen Sayyeda and Qamruzzamab, (2012). Analgesic and Anti-Inflammatory Effect of Ethanol Extract of *Tribulus Terrestris* L. Research Journal of Pharmaceutical, Biological and Chemical Sciences . 3 (4) : 1365

Ka ul, N.; Ag r a wa l, H.; Pa t i l, B.; Ka kad, A. and Dha R. (2005). Application on of stability - indicating HPTLC method for quantitative determination of metadoxine in pharmaceutical dosage form. Farmaco 60 : 351-360.

Lee, HN.; Surh, YJ. (2012). Therapeutic potential of resolvins in the prevention and treatment of inflammatory disorders. Biochem Pharmacol 84(10): 1340-1350

Mahammad ,S.; Maruthi,E.; Bheemewsaraiah,K.; S. Manjunatha,S. and Kodidhela, L. (2012) Effect of *Tribulus terrestris* (L.) on liver in Isoproterenol-Induced Myocardial Infarction**.** International Journal of Research in Biochemistry and Biophysics . 2(4): 10-12

Mona,S.; Mohamed, F. and Wadah, J. (2014). Chromatographic finger print analysis of anti-inflammatory active extract fractions of aerial parts of *Tribulus terrestris* by HPTLC technique Asian Pac J Trop Biomed. 4(3): 203-208

Shraddha Anwikar and Milind Bhitre (2010). Int. J. Ayurveda Res. 1(3), 167–171.

Su, S.; Hua, Y.; Wang, Y. ; Gu, W.; Zhou, W. ; Duan, JA.; Jiang, H. ; Chen, T. and Tang, Y.(2012). J Ethnopharmacol 139: 649-56.