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# Research Article
# Evaluation of Toxicity and Anti-microbial Activity of *Tribulus terrestris* Fruits in Sudan

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## Abstract

**Background and Objective:** *Tribulus terrestris,* a plant of Zygophyllaceae family have been widely used in folk medicine for the treatment of urinary stones, circulatory system problem, intestinal parasitic worms and for skin disorders and many other body ailments. In Sudan, it is traditionally used to remove the urinary tract stones. The present study aimed to evaluate the potential toxicity of *T. terrestris* fruits on Wister rats by using three different doses in 8 weeks. **Materials and Methods:** The rats were allocated into 4 groups, each of 6 rats. Group 2,3 and 4 rats were fed a diet containing different concentrations of *Tribulus terrestris* plant. The mortality and weight gain, sero-biochemical and hematological parameters were recorded in addition to determination of lipid profile and pathological changes. In addition, the anti-microbial activity of *T. terrestris* fruits methanolic extract were tested against two Gram+ve bacteria (*Staphylococcus aureus*, *Bacillus subtitles*), three Gram-ve bacteria (*Salmonella* sp., *Proteus vulgaris*, *Escherichia coli*) and *Candida albicans*. **Results:** The results showed no significant changes observed in all the measured parameters including serum lipid profile, hematological and sero-biochemical parameters, they were not significantly different from the control group. In addition, histopathological examination revealed no lesions observed on the vital organs namely, liver, kidney and intestine. Methanolic extracts from the fruits of *T. terrestris* showed a broad spectrum of anti-microbial activity against tested pathogens. **Conclusion:** The characteristic traits of *T. terrestris* fruits are not toxic and found to be a save alternative to be used in the treatment of various diseases.

Key words: *Tribulus terrestris*, toxicity, antimicrobial activity, lipid profile, Wister rats

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

## INTRODUCTION

There is an increasing interest in various medicinal plants and their bioactive ingredients all over the world especially in developing countries. Herbal drugs have gained importance in recent years for the management of variety of ailments due to their easy access, better compatibility, assumed effectiveness and for economical reasons[1,2]. The consumption of herbal formulations by public have been greatly increased because of the strong believe that plant products are always safe because they are natural, so it is avoid of toxic side effect often associated with synthetic drugs. Recent evidences suggested that some of the plants that have been used for many decades can cause serious side effects and associated with health hazards[3-5]. Therefore, evaluation of the toxicity of medicinal plants is essential for production of effective herbal therapeutics.

*Tribulus terrestris* is an annual plant, commonly known in Sudan as Diraissa and it is widely distributed around the world. It belongs to the Zygophyllaceae family native to warm temperate and tropical regions of the Old World throughout Africa, in southern Europe, in northern Australia and in southern Asia. This plant has been used in traditional medicine for the treatment of various diseases for hundreds of decades. The main active constituents of *Tribulus terrestris* include saponins, flavonoids, alkaloids, amides, glycosides and lignin as demonstrated by various phytochemical studies[6-9]. Modern investigation showed that the chemical constituents steroidal saponins and flavonoids, with the prominent anti-inflammatory and anti-aging activities of *Tribulus terrestris* were the main contributors to the traditional pharmacological activities.

In folk medicine, *Tribulus terrestris* have been used in major cultures in geographic areas like in India, China, Africa and south-eastern Europe to treat vitiligo, urological infections, prostatic hypertrophy and edema as well as diseases of the eyes, abdomen and cardiovascular system[10]. In some countries, *T. terrestris* has been added to herbal mixtures and drugs, which are used to treat sexual dysfunction and enhance sexual performance[11-13]. In Sudan, the fruits of *Tribulus terrestris* (Fig. 1) have been used in the treatment of inflammatory disorders and in nephritis and as a demulcent. Other Biological activities such as aphrodisiac, immunomodulatory, anti-diabetic, hypo-lipidemic, cardiotonic, hepato-protective, antispasmodic, absorption enhancing, analgesic anti-cancer, anti-bacterial and anthelmintic were reported from various *Tribulus terrestris* fruit extracts[14].

Some of the beneficial roles of *T. terrestris* are attributed to its anti-microbial activity[15,16]. The anti-microbial agents are basically important in reducing the spread of infectious diseases. The effectiveness of antibiotics decreased due to the development and spread of resistant pathogens[17]. Therefore, alternative antimicrobial strategies, such as plants and plant-based products are urgently needed. The anti-bacterial activity of *T. terrestris* has been widely studied[18,19]. In previous studies, *T. terrestris* was extracted with different solvents and the results showed that methanolic extracts has the highest inhibition zone for *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus*[20,21].

Fig. 1: *Tribulus terrestris Linn* (fruits)

Although *T. terrestris* fruits, leaf and roots are commonly used in folk medicine in Sudan and other countries in the treatment of various disorders, there is a lack of information about the toxicity and possible side effects of this plant. This study was designed to investigate the toxicity of different doses of *T. terrestris* and to evaluate the effect on growth, organ pathology and hematological and serobiochemical parameters. Furthermore, a screening of methanolic extracts of *T. terrestris* fruits against five pathogenic bacteria and fungi is done in order to test the antimicrobial activity and detect new sources of antimicrobial agents.

## MATERIALS AND METHODS

### Evaluation of *Tribulus terrestris* fruits toxicity on wistar rats

**Plant material:** The fresh and healthy *T. terrestris* fruits (Fig. 1) were collected from botanical garden in Khartoum (April, 2017), Sudan and the plants were identified by Taxonomist. The plant fruits were shade-dried and crushed to powder mimicking the traditional method using manual grinding machine.

Fig. 2: Albino wister rats

**Experimental animals:** Experimental 24, 3-month old both sexes, Wister rats (Fig. 2) with average body weight (90-112 g) were used in this study. The rats were apparently clinically healthy and housed within the premises of Al-Neelain University Animal House under standard husbandry conditions of temperature and light (30±5°C and 12 h light-dark cycle) and fed with the rat diet and water provided *ad libitum*. Animals were acclimatized to the experimental conditions for a period of one week prior to the commencement of the experiment.

**Experimental design:** After one week of acclimation, the rats were divided at random in to 4 groups, each of 6 rats as follows: Group 1 were untreated control and fed normal diet, Group 2 rats were fed a diet containing 2% (w/w) of *T. terrestris*, Group 3 were fed a diet containing 5% (w/w) of *T. terrestris*. Groups 4 were fed a diet containing 10% (w/w) of *T. terrestris* for 2 months.

The body weights of rats were measured weekly for each group. Clinical signs were recorded all through the experimental period. Blood samples for heamatology and serum analysis and tissue samples for histopathology were collected after scarifying all animals from each group under mild chloroform anesthesia.

**Hematological and serobiochemical analysis:** Blood samples for measurements of complete blood count (CBC) were collected in EDTA containing containers and analyzed immediately using Automated Haematology Analyser (Sysmex KX-21, Japan, 1999). Blood samples for serum chemistry analysis were collected in plain containers, centrifuged at 350 rpm for 5 min then the serum were separated, properly labeled and stored at -20°C until used.

Hemoglobin concentration (HB), packed cell Volum (PCV), Red blood cell (RBC), white blood cell (WBC), mean corpuscular Volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were estimated.

Serum was anlayzed for the activities of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) and for concentration of total protein, albumin, globulin, urea and creatinin by using commercial kits (Linear Chemicals, Barcelona, Spain).

**Determination of lipid profile:** Serum lipid profile (total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) were measured by the spectrophotometric methods using commercial diagnostic kits (Biosystem Chemicals, Barcelona, Spain).

**Histological examination:** For histological analysis, necropsy was conducted and all rats were examined to identify gross lesion. Specimens of the liver, kidney and small intestines were collected, immediately fixed in 10% formalin, embedded in paraffin wax. Subsequently, sectioned at 5 µm thick with microtome and stained with haematoxylin and eosin (H and E) using Harris's hemalum. Staining was performed at room temperature and tissues were observed under a light microscope.

**Statistical analysis:** For the analysis of the data, Statistical Package for Social Science (SPSS) was used. The significance of difference between means was compared at each point using Duncan's multiple range tests after ANOVA for one-way classified data[22]. Results were presented as the mean±standard deviation. The $p<0.05$ was considered to indicate a statistically significant difference.

**Anti-microbial activity of methanol extracts of *T. terrestris* fruits**

**Plant extract preparation:** Methanol extract of *T. terrestris* was prepared using maceration procedure. About 150 g of the powdered material (fruits of *T. terrestris*) was extracted with methanol (99.9%) at room temperature for 72 h. Extract was filtered and the solvent was evaporated on the rotary vacuum

evaporator at 72°C under reduced pressure. The extracts were further dried at room temperature after which they were subjected to anti-microbial tests.

**Anti-microbial activity:** The bacterial organisms used in this study included; *Staphylococcus aureus*, *Bacillus subtitles*, *Salmonella* sp., *Proteus vulgaris*, *Escherichia coli* and the fungal organism *Candida albicans* were chosen based on their clinical and pharmacological importance. Nutrient broth was used to prepare the cultures of bacteria and fungi, 3-5 well isolated colonies were picked from appropriately incubated agar cultures, transferred to a sterile culture tube containing 5 mL nutrient broth, incubated at 37°C for the bacteria and 28°C for the fungi for 24 h.

Microbial growth inhibitory potential of the *T. terrestris* were investigated by the agar disk diffusion method as described by CLSI. Anti-microbial activity testing was carried out by using agar well diffusion assay method. *T. terrestris* purified extract was dissolved in dimethyl sulfoxide (DMSO)[23] to make stock solution (100% concentration), then a serial dilution were made at 10, 25 and 50%.

Under sterile conditions swabs were dipped into the broth culture of the organisms. Muller-Hinton Agar plates were streak in three directions to insure complete spread of micro-organisms, the plates then left to stand for about 5 min three equidistant wells of 10 mm in diameter were made on the agar using a sterile cutter and labeled with the cod number of the test crude extract. The disc of standard antibiotic was added in the middle of the plate as positive control and then approximately 20 µL of extracts were dropped into each well which filled them, respectively to fullness. The plates were stored for at least 1 h to allow diffusion of the extract into the agar, while arresting the growth of the tested microbes. The plates were incubated at 37°C for bacteria and 28°C for fungi for 24 h. Anti-microbial activity was determined by measuring diameters of zone of inhibition after 3 replicates, average and mean values were tabulated.

## RESULTS

**Growth changes:** The effects of treatments with diets consisting of 2, 5 and 10% of *T. terrestris* fruits on body weight gains of treated rats were presented in Table 1 and Fig. 3. After 7 weeks of the experiment, there was a significant increase ($p<0.05$) on the body weight of treated groups (2, 3 and 4) when compared to normal control (group 1). No death among the rats recorded along the treatment.

**Hematological changes:** The haematological changes for rats given daily dose of *T. terrestris* fruits at 2% (group 2), 5% (group 3) and 10% (group 4) for 2 months were presented in Table 2. After 2 months of treatment all hematological parameters (Hb, RBC, MCV, MCH, MCHC, PCV, WBC) in all treated groups showed no significant changes compared to control (group 1).

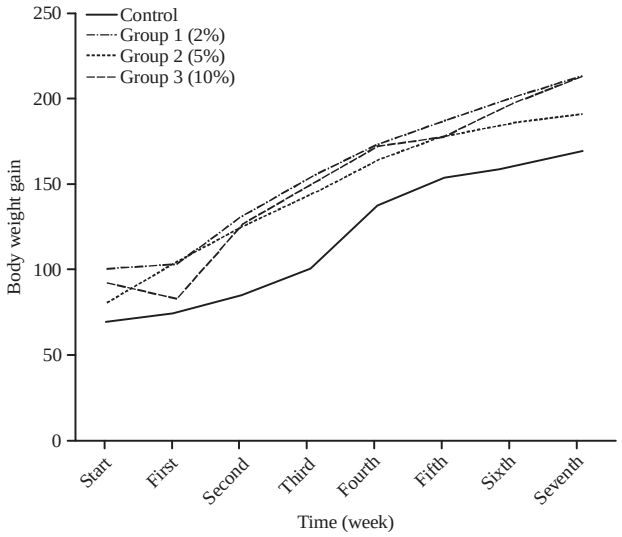


Fig. 3: Body weight and body weight gain in rats given feed *Tribulus terrestris* fruits compared with control for 7 weeks

Table 1: Body weight and body weight gain in rats feed *Tribulus terrestris* (fruits) for seven weeks

| | Body weight gain (g) | | | | |
|---|---|---|---|---|---|
| Groups | Day 0 | Two weeks | Four weeks | Six weeks | Seven weeks |
| Control (untreated) | 90.0±14.4 | 117.0±12.4 | 148.0±21.2 | 180.5±20.5 | 188.5±25.9 |
| *T. terrestris* (2%) | 91.2±12.3 | 132.0±8.9 | 172.5±10.7 | 201.0±11.2 | 212.0±10.1 |
| *T. terrestris* (5%) | 92.0±10.4 | 126.6±9.3 | 164.3±10.4 | 185.3±10.7 | 191.3±9.2 |
| *T. terrestris* (10%) | 91.5±21.5 | 127.0±23.8 | 171.3±25.7 | 197.3±27.9 | 212.0±34.3 |

Values are expressed as Mean±SE, NS: Not significant, *Significant: $p<0.05$

Table 2: Haematological analysis of rats feed *T. terrestris* fruits for 8 weeks

| Parameters | Control (untreated) | *T. terrestris* fruits (2%) | *T. terrestris* fruits (5%) | *T. terrestris* fruits (10%) |
|---|---|---|---|---|
| WBC ($\times 10^3$ mm$^3$) | 9.433±0.92$^{NS}$ | 8.28±0.62$^{NS}$ | 12.23±4.51* | 11.93±4.85* |
| RBC ($\times 10^6$ mm$^3$) | 8.07±0.346$^{NS}$ | 8.48±0.64$^{NS}$ | 8.51±0.273$^{NS}$ | 8.62±0.34$^{NS}$ |
| HGB (g dL$^{-1}$) | 14.30±0.42$^{NS}$ | 14.18±1.13$^{NS}$ | 14.40±0.55$^{NS}$ | 14.63±0.62$^{NS}$ |
| PCV (%) | 44.03±1.00$^{NS}$ | 43.48±3.24$^{NS}$ | 43.73±1.51$^{NS}$ | 44.80±1.86$^{NS}$ |
| MCV (m$^3$) | 54.667±1.25$^{NS}$ | 51.78±1.15$^{NS}$ | 51.50±1.852$^{NS}$ | 52.03±0.74$^{NS}$ |
| MCH (pg) | 17.70±0.40$^{NS}$ | 16.80±0.53$^{NS}$ | 16.867±0.55$^{NS}$ | 16.93±0.28$^{NS}$ |
| MCHC (%) | 32.40±0.23$^{NS}$ | 32.53±0.34$^{NS}$ | 32.9±0.26$^{NS}$ | 32.63±0.088$^{NS}$ |

Values are expressed as Mean±SE, NS: Not significant, *Significant: p<0.05

Table 3: Sero-biochemical analysis of rats feed *Tribulus terrestris* fruits for 8 weeks

| Parameters | Control (untreated) | *T. terrestris* fruits (2%) | *T. terrestris* fruits (5%) | *T. terrestris* fruits (10%) |
|---|---|---|---|---|
| AST (iu) | 310.00±26.8 | 326.00±71.9$^{NS}$ | 359.30±31.8$^{NS}$ | 303.00±13.0$^{NS}$ |
| ALT (iu) | 85.38±8.8 | 74.00±13.8* | 87.30±19.7$^{NS}$ | 85.00±3.0$^{NS}$ |
| ALP(iu) | 259.30±21.1 | 256.70±86.5$^{NS}$ | 242.70±37.0$^{NS}$ | 219.00±20.0$^{NS}$ |
| Total protein (g dL$^{-1}$) | 8.50±0.3 | 7.63±1.3$^{NS}$ | 7.90±0.1$^{NS}$ | 8.20±.04$^{NS}$ |
| Albumin (g dL$^{-1}$) | 3.70±0.1 | 3.40±0.6$^{NS}$ | 3.50±0.1$^{NS}$ | 3.70±.05$^{NS}$ |
| Globulin (g dL$^{-1}$) | 4.80±0.1 | 3.50±0.2$^{NS}$ | 4.40±0.2$^{NS}$ | 4.60±0.4$^{NS}$ |
| Creatinine (mg dL$^{-1}$) | 0.71±0.5 | 0.52±0.1$^{NS}$ | 0.84±0.2$^{NS}$ | 0.82±0.1$^{NS}$ |
| Urea (mg dL$^{-1}$) | 41.00±9.2 | 35.60±4.1* | 43.70±4.8$^{NS}$ | 53.00±0.0$^{NS}$ |

Values are expressed as Mean±SE, NS: Not significant, *Significant: p<0.05

Table 4: Lipid profile analysis of rats feed *Tribulus terrestris* fruits for 8 weeks

| | Parameters | | | |
|---|---|---|---|---|
| Treatment groups | TC (mg dL$^{-1}$) | TG (mg dL$^{-1}$) | HDL-C (mg dL$^{-1}$) | LDL-C (mg dL$^{-1}$) |
| Control (untreated) | 103.7±5.0$^{NS}$ | 91.6±8.1$^{NS}$ | 9.32±6.1$^{NS}$ | 76.06±9.4$^{NS}$ |
| *T. terrestris* (2%) | 120.1±9.0$^{NS}$ | 106.4±54.0$^{NS}$ | 7.80±3.3$^{NS}$ | 93.1±6.8$^{NS}$ |
| *T. terrestris* (5%) | 116.4±5.8$^{NS}$ | 70.6±18.5$^{NS}$ | 8.75±1.77$^{NS}$ | 91.0±2.7$^{NS}$ |
| *T. terrestris* (10%) | 112.6±9.8$^{NS}$ | 95.4±54.0$^{NS}$ | 7.75±3.77$^{NS}$ | 85.8±7.0$^{NS}$ |

TC: Total cholesterol, TG: Triglycerides, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol, SE: Standard error. Values are expressed as Mean±SE, NS: Not significant, *Significant: p<0.05

Table 5: Zone of inhibition of the *Tribulus terrestris* fruits methanolic extracts against the test micro-organism (mm)

| | Mean diameter of inhibition zone (mm) | | |
|---|---|---|---|
| Tested organisms | 10% | 25% | 50% |
| *Escherichia coli* | - | 16 | - |
| *Staphylococcus aureus* | 18 | - | 15 |
| *Salmonella typhi* | 15 | - | - |
| *Bacillus subtilis* | 17 | 15 | - |
| *Proteus vulgaris* | - | 15 | 16 |
| *Candida albicans* | 15 | - | 18 |

-: No inhibition zone

**Sero-biochemical changes:** Sero-biochemical data were summarized in Table 3. There were no significant difference in activity of AST, ALT and ALP or concentration of total protein, albumin and globulin between the control (group 1) and test groups 2, 3 and 4 over the 8 weeks period. Furthermore, no significant changes were observed in the concentrations of Creatinine and Urea compared to the control group.

**Lipid profile changes:** Serum lipid profile analysis was summarized in Table 4. There were no significant differences in lipid profile (triglycerides, high density lipoprotein) for all groups compared with control group.

**Pathological changes:** After 8 weeks of treatment, no significant changes were observed in the liver and small intestine of the rats given daily *T. terrestris* fruits at 2, 5 and 10% of the diet (groups 2-4) compared with rats on the controls (group 1). On microscopy, there was no any change or damage to the 3 sectioned vital organs; liver, kidneys and small intestine (Fig. 4-6).

**Anti-microbial activity of methanol extracts of *T. terrestris* fruits:** *In vitro* antibacterial and anti fungal activities were examined for the methanolic extracts. Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial and antifungal activities were presented in Table 5.

The methanol extract in concentration 50% had an anti-microbial activity against *S. aureus* and *Proteus vulgaris* with growth inhibition zones 15 and 16 mm, respectively. The methanol extract in concentration 25% showed an anti-microbial activity against *E. coli*, *Bacillus subtilies* and *Proteus vulgaris* with growth inhibition zones 16, 15 and 15 mm, respectively. The growth inhibition zone measured for

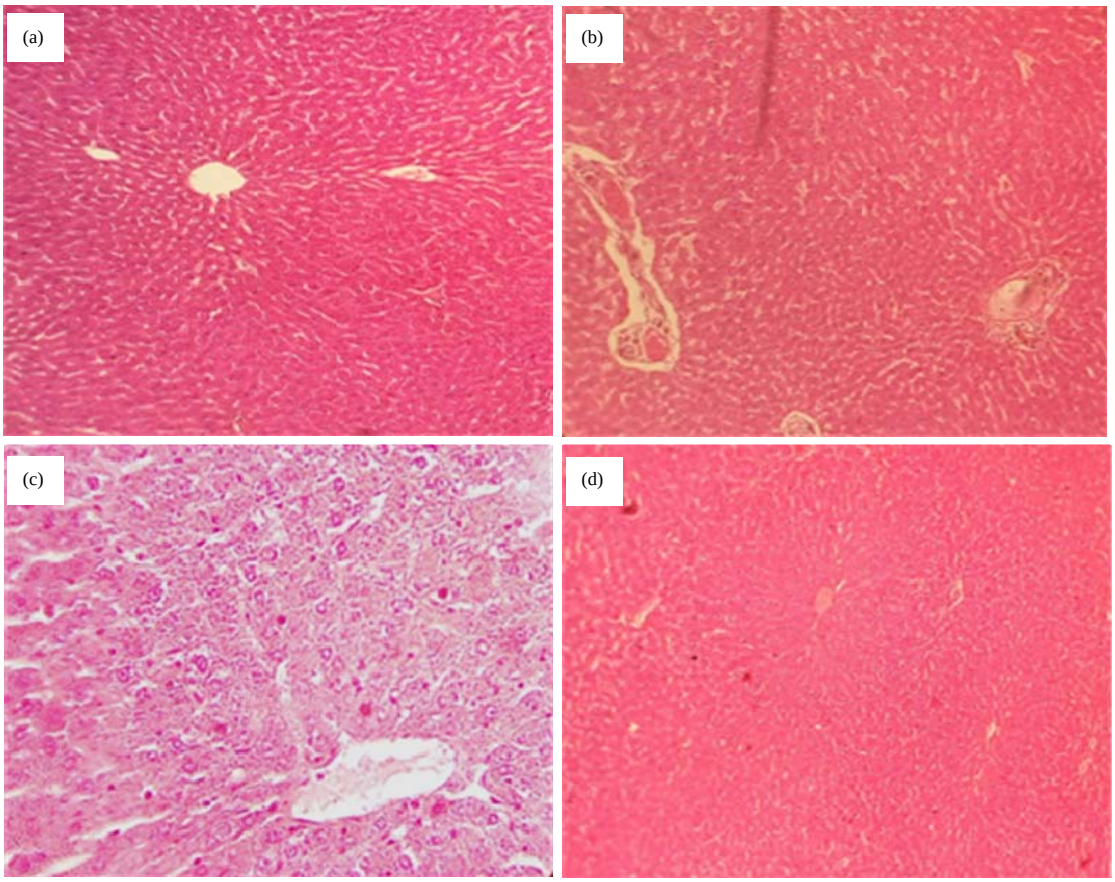


Fig. 4(a-d): Liver of rats given *T. terrestris* in diet for 8 weeks, (a) Normal hepatic structure in the control group, (b) Necrosis of the centrilobular hepatocytes in rats received 2% of *T. terrestris*, (c) Liver of rat receiving daily *T. terrestris* fruits at 5% of the diet showing no lesions were observed and (d) Liver of rat receiving daily *T. terrestris* fruits at 10% of the diet showing no lesions were observed (H and E staining 100x)

Table 6: Mean diameter of inhibition zone (MDIZ) of control against tested micro-organisms (mm)

| | Standard antibiotics | |
|---|---|---|
| Tested organisms | Azithromycin | Nystatin |
| *Escherichia coli* | 16 | - |
| *Staphylococcus aureus* | 20 | - |
| *Salmonella typhi* | 18 | - |
| *Bacillus subtilis* | 22 | - |
| *Candida albicans* | - | 25 |

-: No inhibition zone

the 10% methanolic extract against *S. aureus*, *Salmonella* spp., *Bacillus subtilies* and *Candida albicns* are 18, 15, 17 and 15 mm, respectively, Fig. 7 showed inhibition zone (18 mm) of *Tribulus terrestris* methanolic extract (10%) against *Staphylococcus aureus*.

The antimicrobial activities of control antibiotics were estimated against the tested pathogens as shown in Table 6. *Azithromycin* had antibacterial activity against tested bacteria with inhibition zones 20 mm for *S. aureus*, 16 mm for *E. coli*, 22 mm for *B. subtitles* and 18 mm for *Salmonella* sp., *Nystatin* had activity against *C. albicans* with inhibition zone 25 mm.

## DISCUSSION

Species of the genus of *Tribulus* are perennial herbs mainly distributed in both tropical and mild temperate region. These plants are used in traditional medicine formulas for multiple purposes as herbal medicine. The phytochemical studies on *T. terrestris* revealed the presence of numerous bioactive phytochemicals like saponins, glycosides, flavonoids, alkaloids, glycosides, phytosteroids and other constituents. Although the *T. terrestris* fruits are used in traditional medicine of several countries, there is no toxicological information on rodents and other species and the clinical trials with *T. terrestris* are scarce.

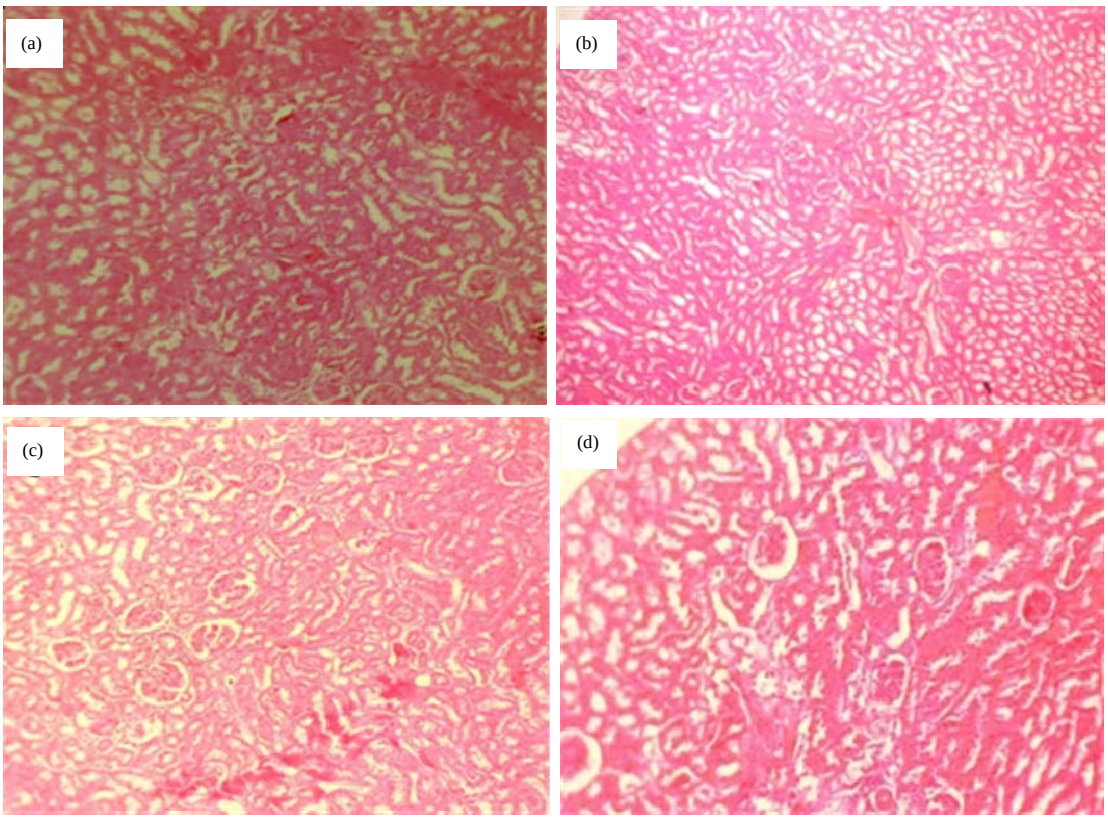


Fig. 5(a-d): Kidneys of rats given *T. terrestris* in diet for 8 weeks, (a) Represent kidney of control group, (b) Kidney of rats received low dose (2%) of *T. terrestris* fruits daily showing no change in Glomerular tubules comparing to control group, (c) Kidney of rats receiving daily *T. terrestris* fruits at 5% of the diet show change of Glomerular tubules comparing to control group and (d) Kidney of rats receiving daily *T. terrestris* fruits at 5% of the diet; it shows no change in the Glomerular comparing to control group (H and E stained 100x)

From this *in vivo* study, it can concluded that the *T. terrestris* fruits in different doses 2, 5 and 10% in diet for 8 weeks on Wistar rats induce a significant increase on the body weight gains when compared to control group, this indicated that, the fruits may interfere with growth processes. No significant changes were observed in the biological parameters like serology and no damage in the vital organs based on microscopic observation and alterations in components synthesized by liver like enzymes and proteins. Even the higher concentration doesn't affected or significantly changed parameters and organs. In histopathological study it was found that Group 2, 3 and 4, Group 3 were normal, the intestine and kidney and liver in all groups were normal in this study and that is disagreed with[24] that founded *T. terrestris* inhibit WBC count and had effect on liver and kidney that it cause hepatocellular degeneration, renal tubular necrosis.

In addition, *T. terrestris* fruits which is used intensively as kidney stones clearance especially in traditional Sudanese medicine had no toxic effects on kidney function and had no pathogenic effect on kidney structures, so it's likely to have a protective effect on kidney. This was in agreement with a previous study[25] that confirmed the positive effect of the plant on the kidney tissues and function, as indicated by observations on the urea and creatinine levels. Also there is an increase in body weights of experimental groups comparing to control group and that agreed with another study[26] in which they reported that *T. terrestris* has a role in muscle-building.

Previous studies on *T. terrestris* toxicity had been conducted only among animals. The acute toxicity of methanolic extracts was previously investigated, 2 g $kg^{-1}$ was given orally to five mice for a period of 14 days and there were no toxic symptoms or mortality observed among the animals[27]. Another, *in vitro* study was conducted to evaluate the geno-toxic potential of *T. terrestris* extracts. Toxicity of *T. terrestris* extracts at 3-2400 µg $mL^{-1}$ was assessed by

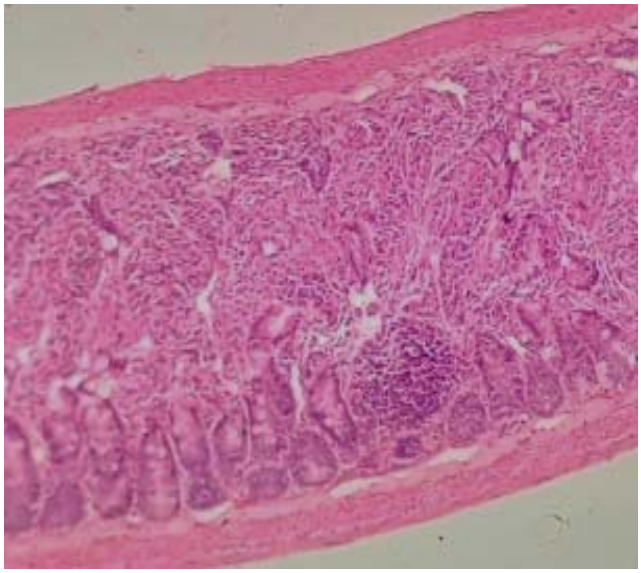

Fig. 6: Intestine of rat receiving daily *Tribulus terrestris* fruits at 2% of the diet for 8 weeks showing no lesions observed in intestinal epithelium (H and E x100)

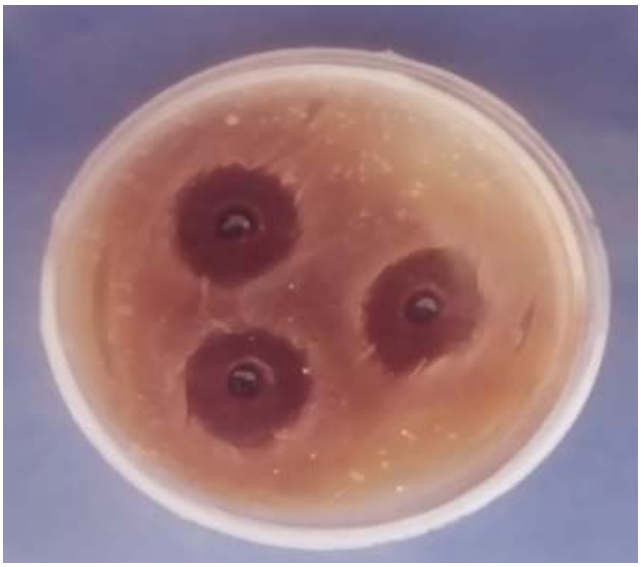

Fig. 7: Inhibition zone (18 mm) of *Tribulus terrestris* methanolic extract (10%) against *Staphylococcus aureus*

Comet assay in a rat kidney cell line and by Ames assay in *Salmonella typhimurium*, results showed the methanol extract of *T. terrestris* had relatively higher geno-toxic activities (2400 mg $mL^{-1}$ METT, tDNA%: 11.43) and cytotoxic activities ($IC_{50}$ = 160 mg $mL^{-1}$) than water and chloroform extracts[28]. Symptoms of severe damage of cardiac muscle, liver and kidney were noted in native goats and sheep when their daily meals contained 80% fresh plants[29,24].

The *T. terrestris* fruits showed no toxic effects on rats in this study, this is maybe due to that secondary metabolites of fruits had low ability to cause toxic effects or they were low molecular weight compounds so easy filtrate through kidneys and out in urine without causing any toxic effect. Other reason is that the metabolisms of the fruits metabolites may produce less toxic compounds that are easily excrete through kidney.

Recently, a great attention is directed toward the isolation and study of natural products that active against bacteria and fungi that cause diseases. Anti-microbial activity of *T. terrestris* in methanol extract in 3 concentrations (10, 25 and 50%) were examined against 5 pathogenic bacteria (*Staphylococcus aureus*, *Bacillus subtitles*, *Escherichia coli*, *Salmonella* sp. and *Proteus vulgaris*) and fungi (*Candida albicans*). The result revealed an antibacterial activity against both Gram-positive and negative bacteria and *Candida albicans* which was agreed with an earlier study[24]. The activity of methanol extract differed according to concentrations and species, the 10% is more active in *S. aureus* (the zone of inhibition is18 mm). For standard antibiotic the results have been showed that the Azythromycin and Nystatin had a strong anti-microbial activity against tested bacteria and fungi. So the use of methanol extract of *T. terrestris* as an alternative source for antibiotic is appropriate.

Furthermore, the effect of *T. terrestris* in lipid profile was evaluated in this study. Treatment with *T. terrestris* showed no significant change in the serum lipid levels, this was in contrast with a previous study stated that saponins could significantly lower the levels of serum TC, LDL[30].

## CONCLUSION

This study concluded that *T. terrestris* is not toxic and safe to be used traditionally for treatment of diseases. For more confirmation of the aspects concerning safety of this plant, more researches on human clinical studies to support the traditional use are required. The methanolic extract of that plant had promising value against bacteria and fungi. Although *in vitro* study, this should be taken into consideration for the development of new herbal medicine using *T. terrestris*

## SIGNIFICANCE STATEMENT

In spite of the intensive uses of plant in developing countries, especially rural areas, there is no standardized doses system in traditional medicine practice. Experimental research

must be carried out to provide scientific validation of this traditional knowledge in Sudan. In Sudan, *Tribulus terrestris* have been used in the treatment of various disorders and there is a lack of information about its safety, efficacy and possible side effects. In this article, the toxicity of *Tribulus terrestris* fruits was studied, as they have been extensively used in Sudan, in an effort to the ultimate demonstration of therapeutic usefulness of the plant. This study will be taken into consideration for the development of new herbal medicine using *T. terrestris*.

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