

Study the Biological Activities of *Tribulus Terrestris* Extracts

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Abstract—In this study the extracts of the Iraqi herb *Tribulus terrestris* (Al-Hassage or Al-Kutub) was done by using of polar and non polar solvents, then the biological activity of these extractants was studied in three fields, First, the antibacterial activity (in vitro) on gram positive bacteria (*Staphylococcus aureus*), and gram negative bacteria (*E. coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebsiella*), all extracts showed considerable activity against all bacteria. Second, the effect of extracts on free serum testosterone level in male mice (in vivo), the alcoholic, and acetonitrilic extracts showed significant ($P < 0.05$) increase in free serum testosterone level, and we found that the extracts contained compounds with less genotoxic effects in mice germ cells. 3rd, was to study the effect of methanolic extract of *T. terrestris* in diabetes management.

Keywords—Genotoxic, germ cells, tribulus terrestris, testosterone.

I. INTRODUCTION

TRIBULUS TERRESTRIS is a natural herb used for treating many diseases like hypertension[1]. It is a member of the Zygophyllaceae family, and an annual herb found in many tropical and moderate areas of the world, including the U.S. and Mexico, the Mediterranean region, and throughout Asia[2]. *Tribulus terrestris*, is also known as Puncture Vine, It contains steroidal saponins, and act as a natural testosterone enhancer. *Tribulus terrestris* increases testosterone through increasing lutenizing hormone (LH). There is good confidence that *Tribulus terrestris* is useful as a sexual enhancement herb[3]. In Iraq *T. terrestris* is used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, anti-hypertensive, diuretic, lithon-triptic and urinary anti-infectives[4,5].

Mainstream medicine is increasingly receptive of the use of antimicrobial and other drugs derived from plants, as traditional antibiotics become ineffective and because of the rapid rate of plant species extinction. There is a feeling among

natural-products chemists and microbiologists alike that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at risk of being lost irretrievably[6].

The aim of this work was to study the biological activity of the Iraqi herb *Tribulus terrestris* as antibacterial and testosterone enhancer moreover study genotoxic effects in germ cells of mice.

II. EXPERIMENTAL

A. Extraction Procedure

Tribulus Terrestris were collected from natural habitats during flowering. Air dried plant sample rinsed with water and dried. After evaporation of the solvent, the residues were powdered (250 g) and extracted with 500ml, 70% ethanol (or methanol, or acetonitril, or hexane) in a soxhlet apparatus and the extracts were evaporated to dryness by a rotary evaporator.

B. Agar Diffusion Assays

Different strains of bacteria were used which are: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella*, *Proteus vulgaris* and *Staphylococcus aureus*. All strains were collected from Biochemical division, Department of applied science, University of Technology. The identity of all the strains were confirmed. A bacterial suspension was prepared and added to the sterilized medium before solidification. The media with bacteria was poured into sterilized Petri dishes under aseptic condition. Different weights of the extractants (alcoholic, mthanolic, acetonitrilic, and hexanic, 0.02 M) in N,N-dimethylformamide (DMF) solvent were placed on the surface of the culture and incubated at 37 °C for 24 hours. After incubation the average of inhibition zones was recorded[7-8].

C. Determination of Free Serum Testosterone

The level of free serum testosterone was measured according to Enzyme-linked immunosorbant assay [9].

D. Animals

Healthy, adult 20 male mice weighing 25-30g, aged 2-3 months were used in this study. The animals had free access to a standard commercial diet and water, they were kept in rooms maintained at 25-27°C. The animals were divided randomly into different groups; each group consisted of six male mice. First Step: Control group 2 male mice orally treated with distilled water (2 ml/kg) three times per week for 45 days.

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Second Step: Group 2 (3 male mice) orally treated with 70% alcoholic extract 20 mg / kg body weight three times per week for 45 days.

Third Step: Group 3 (3 male mice) orally treated with methanolic extract 20 mg / kg body weight three times per week for 45 days. Forth Step: Group 4 (3 male mice) orally treated with acetonitrilic extract 20 mg / kg body weight three times per week for 45 days. Fifth Step: Group 5 (3 male mice) orally treated with hexanic extract 20 mg / kg body weight three times per week for 45 days. Sixth Step: Group 6 (3 male mice) orally treated with Bulgarians Tribulus terrestris extracts 50 mg / kg body weight three times per week for 45 days. Seventh Step: Group 7 (3 male mice) orally treated with methyltestosterone 3 mg / kg body weight three times per week for 45 days. Genotoxicity assay, the chromosome preparation of germ cells of mouse was done according to air drying method for meiotic preparation[10].

III. RESULTS AND DISCUSSION

A. Antimicrobial Activity of Tribulus Terrestris Extracts

The bacterial activity of *T. terrestris* varies depending on the origin and plant's part used. The ethanolic extract of the fruit and leaves of Indian herb has activity against *E. coli* and *S. aureus*, but ethanolic extract of *T. terrestris* from Yeman has no detectable anti-bacterial activity, against any of the reference bacteria. The methanolic extract of the same herb grown in Iran has anti-bacterial activity. The activity is reported due to spiroaponins, present in the herb[11].

1: The determination of the MIC

(Minimum inhibition concentration) by means of the agar diffusion assay (Table I) showed that 4 plant extracts tested exhibited an antimicrobial effect against Gram positive bacteria, *Staphylococcus aureus* and Gram negative, *Klebsiella*, *Proteus vulgaris*, *Pseudomonas* and *E. coli*.

TABLE I

THE MIC VALUES IN MG/ML OF *T. TERRESTRIS* EXTRACTS IN AGAR DIFFUSION ASSAY

Extractants	S	E	P	Pa	K
70% Ethanolic	3	3	2	2	3
Methanolic	3	3	3	2	3
Acetonitrilic	2	2	2	2	2
Hexanic	5	5	6	5	7
<i>E=Escherichia coli, Pa=Pseudomonas aeruginosa, K= Klebsiella pneumoniae, P=Proteus vulgaris and S=Staphylococcus aureus</i>					

2: The Antibacterial activity of *T. terrestris* extracts

The antibacterial activities of the plant extracts were evaluated by measuring the inhibition zone observed around the tested materials.

In agar diffusion assay, the hexanoic and methanolic extracts of the plant showed considerable activity against all tested bacteria (Table II).

TABLE II
THE ANTIBACTERIAL ACTIVITY OF *T. TERRESTRIS* EXTRACTS AGAINST BACTERIA IN AGAR DIFFUSION ASSAY COMPARED WITH GENTAMYCIN

Extractants	Bactera (Inhibition zone diameter (mm))				
	S	E	P	Pa	K
70% Ethanolic	24.2	20	21	20	22.5
Methanolic	23.2	17.3	20.7	20.5	20.5
Acetonitrilic	24.8	15.7	17.5	20.5	19.5
Hexanic	13	11.5	13.3	11.5	9.5
Gentamycin	15	15	17	18	16
<i>E=Escherichia coli, Pa=Pseudomonas aeruginosa, K= Klebsiella pneumoniae, P=Proteus vulgaris and S=Staphylococcus aureus</i>					

B. Biological Activity on Male Mice

1: Effect *T. terrestris* extracts on free serum testosterone level in male mice

T. terrestris has stimulating effect on spermatogenesis by increase in the amount of Luteinizing Hormone (LH) produced by Pituitary gland, which stimulate the secretion of male hormone 'Testosterone', resulting in significant improvement in quality and quantity of sperm. [12-15]. In the present work *tribulus terrestris* extracts show increasing of free serum testosterone level in male mice (Table III).

TABLE III
FREE SERUM TESTOSTERONE LEVEL IN MALE MICE (NG/ML)

Extractants	Free serum testosterone level in male mice (ng/ml)
Control	9.1
70% Ethanolic	21.1
Methanolic	24.2
Acetonitrilic	23.9
Hexanic	10.7

2: Cytotoxic activity of *T. terrestris* extracts

T. terrestris of different regions (Bulgaria, China and India) and different parts of plants (stem and fruit) shows that only the spiro compounds exhibit remarkable activity. The inhibitory effect of saponin mixture from Chinese origin on Bcap37 breast cancer cell has potent inhibitory effect. [16].

The spontaneous frequency of chromosomal aberration (CAs) in mous germ cells was (0.18 ± 0.06) which represents a control Table IV. The results of this experiment indicate that (0.2, 0.4, 0.8) mg/ mL of methyltestosterone can increase CAs frequencies (Ring chromosome, chromosome break and chromatid break) more than methanolic, ethanolic extracts and also tribestane which reached to (0.36, 0.42, 0.44). These results were significantly different (P<0.05) from the control. The chromosomal aberration induction by methyltestosterone is not fully understood. Suggested that the inhibition of chromosomal protein synthesis might cause a weakening of chromosomal backbone and subsequent chromosomal aberration[17] or may be related to the testicular damage as manifested by reduced testicular volume, elevated FSH and LH protein[18]. Several chemotherapeutic such as fludarabin, cyclophosphamide can cause these damage[19].

TABLE IV
EFFECTS OF TRIBULUS T. EXTRACTS, METHYLTESTOSTERONE AND
TRIBESTAN ON CHROMOSOMAL ABERRATIONS IN GERM CELLS OF MICE

Extractants	T	R	Cb	Cd
Control	0.18±0.06	0.02±0.005	0.08±0.06	0.08±0.04
Methanolic.				
0.2mg/mL	0.16±0.02	0.05±0.01	0.04±0.01	0.07±0.01
0.4 mg/mL	0.23±0.04	0.04±0.01	0.08±0.01	0.11±0.02
0.8 mg/mL	0.26±0.05	0.05±0.05	0.08±0.05	0.13±0.05
Ethanollic				
0.2mg/mL	0.2±0.04	0.18±0.01	0.06±0.03	0.07±0.02
0.4 mg/mL	0.27±0.08	0.11±0.03	0.12±0.02	0.04±0.02
0.8 mg/mL	0.15±0.03	0.00±0.00	0.15±0.01	0.07±0.01
Methyltest.				
0.2mg/mL	0.36±0.06	0.12±0.03	0.08±0.02	0.16±0.01
0.4 mg/mL	0.42±0.08	0.12±0.02	0.12±0.02	0.18±0.04
0.8 mg/mL	0.44±0.07	0.04±0.01	0.21±0.01	0.19±0.05
Tribestan				
0.2mg/mL	0.28±0.19	0.08±0.01	0.08±0.05	0.12±0.01
0.4 mg/mL	0.38±0.13	0.05±0.01	0.12±0.05	0.21±0.07
0.8 mg/mL	0.15±0.07	0.05±0.01	0.04±0.01	0.06±0.03
	T=Total (mean ±SE), R=Ring chromosome (mean ±SE), Cb=Chromosome break (mean ±SE), Cd= Chromatid break (mean ±SE), SE = Standard error			

The extracts of Iraqi *tribulus terrestris* after seven days of treatment by alcoholic, extract, water extract non significant decrease in CAs compared with control, these extracts contain many compounds which may increase the activity of the detoxification enzymes such as superoxide dismutase and glutathione-S-transferase that scavenging free radicals from the cells[20].

3: Effect of methanolic extract of *T. terrestris* in diabetes management

50 mg/kg body wt. concentration of methanolic extract of *T. terrestris* produces significant decrease in blood glucose level, after 4 and 6 hours of treatment as compared to untreated diabetic mice. After 4 and 6 hours of treatment, the percent of reduction in blood glucose level produced by *T. terrestris* (43±4.1), (41±3.4). After three weeks of treatment, blood glucose level in diabetic mice treated with *T. terrestris* decreases (60%) to below normal level. Treatment of diabetic mice by *T. terrestris* extract resulted in significant decrease in serum triglycerides TAG, total cholesterol TC and low density lipoprotein cholesterol LDL as compared to untreated diabetic.

IV. CONCLUSION

The solvent extracts of *T. terrestris* showed good antibacterial activity against Gram positive and Gram negative bacteria. Methanolic extract showed very good activity as testosterone producer in serum tested animals. In addition the methanolic extract of *T. terrestris* produces significant decrease in blood glucose level of diabetic mice.

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REFERENCES

- [1] R. A. Mothana, and U. Lindequist. Antimicrobial activity of some medicinal plants of the island Soqatra. *J. Ethnopharmacol.*, 2005, **96**(1-2):177-181.
- [2] K. Abeywickrama, and G. A. Bean. Toxicogenic *Aspergillus flavus* and aflatoxins in Sri Lankan medicinal plant material. *Mycopa-thologia.*, 1991, **113**: 187-190.
- [3] K. Gauthaman, A. P. Ganesan, and R. N. Prasad. "Sexual effects of puncturevine (*Tribulus terrestris*) extract (protodioscin): an evaluation using a rat model". *Journal of Alternative and Complementary Medicine.*, 2003, **9** (2): 257-265.
- [4] S. H. Majeed, and M. J. Mahmood. Herbs and Medicinal Plants in Iraq between Traditional Medicine and Scientific Research. 1st Ed. Baghdad: Dar Al-Thawra for Publishing., 1988, p. 40. (in Arabic).
- [5] S. Aldein. Medicinal Herbs. 1st Ed. Baghdad: Dar Al-Shoun Al-Thaqafia Al-Aama for Publishing.1986, p. 70. (in Arabic).
- [6] M. M. Cowan. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 1999, **12**(4): 564-582.
- [7] O. Kandil, N. M. Radwan, A. B. Hassan, A. M. Amer, and H. A. El-Banna. Extracts and fractions of *Thymus capitatus* exhibit antimicrobial activities. *J Ethnopharmacol.* 1994, **44**(1):19-24.
- [8] D. McCan, L. Kirkis. Evaluation of Free Testosterone in serum. *J Clin Immunoassay.* 1985, **8**: 234-6.
- [9] M. R. Atlas, E. Alfres, and C. Lawrence "Laboratory Manual Experimental Microbiology" Mosby- Year Book, Inc. 1995.
- [10] E. Evans, G. Breckon, and C. Ford. Air drying method for meiotic preparation from mammalian testes. *Cytogenetics* 1964, **3**: 284-294.
- [11] E. Bedir, A. Khan. New steroidal glycosides from the fruits of *Tribulus terrestris*. *Pharmazie* 2000, **57**:491-3.
- [12] Y. Wan, K. Ohtani, R. Kasai, and K. Yamasaki. Steroidal saponins from fruits of *Tribulus terrestris*. *Phytochemistry* 1997, **45**:811-7.
- [13] A. G. Brown, M. D. Vukovich, E. R. Martini, L. M. Kohut, W. D. Frank, DA Jackson, *et al* . Endocrine and lipid responses to chronic androstenediol-herbal supplementation in 30 to 58 year old men. *J Am Coll Nutr* 2002, **20**:520-8.
- [14] K. M. Arsyad. Effect of protodioscin on the quality and quantity of sperms from males with moderate idiopathic oligozoospermia. *Medica* 1996, **22**:614-8.
- [15] S. K. Bhattacharya. National conference on Recent trends in Spice and Medicinal Plants Research. Calcutta. India: April 1998, p. 652-4.
- [16] B. Sun, W. Qu, and Z. Bai. The inhibitory effect of saponins from *Tribulus terrestris* on Bcap-37 breast cancer line *in vitro*. *Zhong Yao Cai* 2003, **26**:104-6.
- [17] Castoldi, G.; Scapoli, P.; and Spanedda, R. (1969). Chromosomes and chloramphenicol. *Arch. Ital. Patol. Clin. Tumori*, **12**:117-141.
- [18] E.H. Al-Neimy, Biological effects of Capparis spinosa and Rumex acetosella extracts on animal and human normal and tumor cells. Ph.D. thesis, college of science, 2007, Al-Nahrain University.
- [19] R. Chatterjee, G. A. Haines, D. M. Perera, A. Goldston, and L. D. Morris. Testicular and sperm DNA damage after treatment with fludarabine for chronic lymphocytic leukemia. *Hum. Reprod.* 2000, **15**: 762-766.
- [20] Z. y. Wang, S. J. Chen, Z. C. Zhon, M. Athar, W. A. Khan, D. R. Bickers, and H. Muthar. Antimutagenic activity of green tea polyphenols. *Mut. Res.*, 1989, **223**: 273-285.