Study the biological activities of Tribulus terrestris extracts

دراسة الفعالية الحيوية لمستخلصات نبات الحسج (أو القطب)

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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 two field. First, the antibacterial activity *invitro* on gram positive bacteria (*Staphylococcus aureus*), and gram negative bacteria (*E. coli, Proteus vulgaris, Pseudomonas aerugiuosa, and Klebsiella pneumonia*), all extracts showed considerable activity against all bacteria. Second, the effect of extracts on free serum testosterone level in male mice *invivo,* 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.

المستخلص:

في هذه الدراسة تم استخلاص نبات الحسج العراقي بمذيبات قطبية ولا قطبية ومن ثم دراسة التأثير الحيوي لهذه المستخلصات باتجاهين هما الأول: دراسة الفعالية المضادة للبكتريا (للغرام الموجب *Staphylococcus aureus* والغرام السالب *E. coli, Proteus vulgaris, Pseudomonas aerugiuosa, and Klebsiella*) , حيث اظهرت النتائج أن لكل المستخلصات فعالية مضادة للبكتريا . أما الاتجاه الثاني: دراسة تأثير المستخلصات على مستوى التستوستيرون الطبيعي في ذكور الفئران حيث أظهرت النتائج إن للمستخلصات الكحولي والاسيتونايتريلي تأثير في زيادة مستوى التستوستيرون الطبيعي في ذكور الفئران .

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.

Experimental

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.

Soxhlet. *Tribulus terrestris* was extracted in a Soxhlet extractor for 24 hours at a maximum temperature of 65^{o}C, in the proportion of 25g of *Tribulus terrestris* to 5L. of 70% ethanol (or methanol, or acetonitril, or hexane).

Analytical procedures

Yield of *Tribulus terrestris*extracts. All extracts were evaporated to dry and weighed to obtain the yield. The results were given as a percentage of the original weight of crude *Tribulus.*

Thin Layer Chromatography. The analysis was performed on precoated (20cm length, 20cm width, and 0.25mm thickness) TLC plates K6F silica gel 60A purchased from Whatman, USA. The solutions are concentrated to 2 ml., 10 µ litter of this solution is applied on the TLC plate, to establish the R_f value. Detection of the spots was done by the use of spraying Vanillin sulphuric acid reagent.

Agar diffusion assay

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 was 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 ^{o}C for 24 hours. After incubation the average of inhibition zones was recorded [7-8].

Determination of Free Serum Testosterone:

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

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.**

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.**

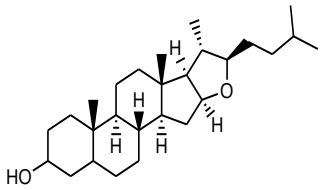
Fifth Step: **Group 5 (3 male mice)** orally **treated with** hexanic *extract* 20 mg / kg body weight **three times per week for 45 days.**

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].

Results and Discussion:

Thin Layer Chromatography . Table (2) shows the TLC analysis results, and indicates the R_f value for the Acetonitrili extractant.

Extractants	**Mobile phase**	**R_f value**	**Name**	**Structures**
Acetonitrilic	**Acetonitrile:water(80:20)**	**9cm**	**Furostanol**	

The determination of the MIC (Minimum inhibition concentration) by means of the agar diffusion assay Table (2) showed that 4 plant extracts tested exhibited an antimicrobial effect against Gram positive bacteria, *Staphylococcus aureus* and Gram negative, *Klebsiella, Proteus vulgasis, Pseudomonas* and *E. coli.* The antibacterial activities of the plant extracts were evaluated by measuring the inhibition zone observed around the tested materials.

Table (2): The MIC values in mg/mL of *T. terrestris* extracts in Agar diffusion assay.

Extractants	Bactreia				
	***Staphylococcus aureus*(G +ve)**	***Escherichia coli* (G -ve)**	***Proteus vulgasis*(G -ve)**	***Pseudomonas aerugiuosa* (G -ve)**	***Klebsiella*(G -ve)**
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**

The 70% ethanolic and methanolic extract of *T. terrestris* has activity against *Staphylococcus aurues, Klebsiella and Escherichia coli,* but Hexanic extract of *T. terrestris* has less detectable anti-bacterial activity, against any all kinds of bacteria. Table (3).

Table (3): Antibacterial activity of T. terrestris extracts against bacteria in Agar diffusion assay compared with Gentamycin .

Extractants	*Diameter (mm) of zone of inhibition*				
	***Staphylococcus aureus*(G +ve)**	***Escherichia coli* (G -ve)**	***Proteus vulgasis*(G -ve)**	***Pseudomonas aerugiuosa* (G -ve)**	***Klebsiella* (G -ve)**
70%Ethanolic	**24.2**	**20**	**21**	**20**	**22.5**
Methanolic	**23.2**	**17.3**	**20.7**	**20.5**	**21.5**
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**

Biological activity on male mice:

T. terrestris extract improves the body's ability to build muscle mass and strength by promoting the production luteinizing hormone, thereby stimulating the secretion of testosterones, resulting in the development of male-like characters (i.e. strong muscles and strength) with increase in sex drive, as well as production of red cells, contributing to improvement in blood circulation and good oxygen transport. [11-16].

Table (4): 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**

The spontaneous frequency of chromosomal aberration (CAs) in mous germ cells was (0.18 ± 0.06) which represents a control table (4). The results of this experiment indicates 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]. 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 andglutathione-S-transferase that scavenging free radicals from the cells[20].

Table (5): Effects of *Tribulus terrestris* extracts, Tribestan & Methyl Testosterone on chromosomal aberrations in germ cells of mice.

	Total (mean ±SE)	Ring chromosome (mean ±SE)	Chromosome break(mean ±SE)	Chromatid break (mean ±SE)
Control	**0.18± 0.06**	**0.02 ± 0.005**	**0.08±0.06**	**0.08 ±0.04**
Methanolic extr.	**0.16±0.02**	**0.05±0.01**	**0.04±0.01**	**0.07±0.01**
0.2mg/mL	**0.23±0.04**	**0.04±0.01**	**0.08±0.01**	**0.11±0.02**
0.4 mg/mL	**0.26±0.05**	**0.05±0.05**	**0.08±0.05**	**0.13±0.05**
0.8 mg/mL				
70% Ethanol.ext	**0.21 ±0.04**	**0.18±0.01**	**0.06±0.03**	**0.07±0.02**
0.2 mg/mL	**0.27±0.08**	**0.11±0.03**	**0.12±0.02**	**0.04±0.02**
0.4 mg/mL	**0.15 ±0.03**	**0.00±0.00**	**0.15±0.01**	**0.07±0.01**
0.8 mg/mL				
Tribestan	**0.28±0.19**	**0.08±0.01**	**0.08±0.05**	**0.12 ±0.01**
0.2mg/mL	**0.38±0.13**	**0.050±0.01**	**0.12±0.05**	**0.21±0.07**
0.4 mg/mL	**0.15± 0.07**	**0.05±0.01**	**0.04±0.01**	**0.06±0.03**
0.8 mg/mL				
Methyltestosterone	**0.36±0.06**	**0.12±0.03**	**0.08±0.02**	**0.16±0.01**
0.2mg/mL	**0.42±0.08**	**0.12±0.02**	**0.12±0.02**	**0.18±0.04**
mg/mL	**0.44±0.07**	**0.04±0.01**	**0.21±0.01**	**0.19±0.05**
0.8mg/mL				

SE = Standard error

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