Review Article

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Sexual Behavior of *Tribulus Terrestris*

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ABSTRACT

Abbreviations: TLC: Thin Layer Chromatography; BP: Blood Pressure; STT: Saponins from Tribulus Terrestris; MSH: Melanocyte Stimulating Hormone

Introduction

Many of today's popular dietary supplements come from plants that have been used medicinally since ancient times. One of these botanicals is *Tribulus terrestris*, which is purported to have a variety of health benefits, including reduced blood sugar, cholesterol, altered hormone levels and increased sexual function and libido. *Tribulus terrestris* is normally distributed in tropical and subtropical countries in Asia, Africa, south Europe, North Australia and introduced in new world tropics. In UAE common and widespread in Urban areas, roadsides and depressions receiving runoff water (Figure 1) [1]. In UAE the plant is used by some healers as an aphrodisiac, diuretic and hypotensive also used to treat dysuria; it is soothing, analgesic, diuretic, tonic, against colic . The leaves of the plant used to treat enlarged spleen, puerperal fever, sores, diarrhea, nervous exhaustion and cramps, normally the whole plant is used. A transverse section of the stem from the periphery to the pith shows the following: an epidermal layer consisting of small ovoid cells with thick cell walls and the epidermis is covered with thick cuticle.

Figure 1: Tribulus Terrestris.

Attached to the epidermis are numerous one-celled covering trichomes that have comparatively wide lumens and thick cell walls. The epidermis is encircling several layer of cortical colenchyma (about 6-7 layers); the inner layers enclose groups of lignified fibres that have thick cell walls and narrow lumens. The cortex encircles phloem tissues, heavily lignified xylem tissues (vessels, trachieds and fibres) and at the centre are the nearly rounded parenchyma cells of the pith which occupy a wide zone (Figure 2).

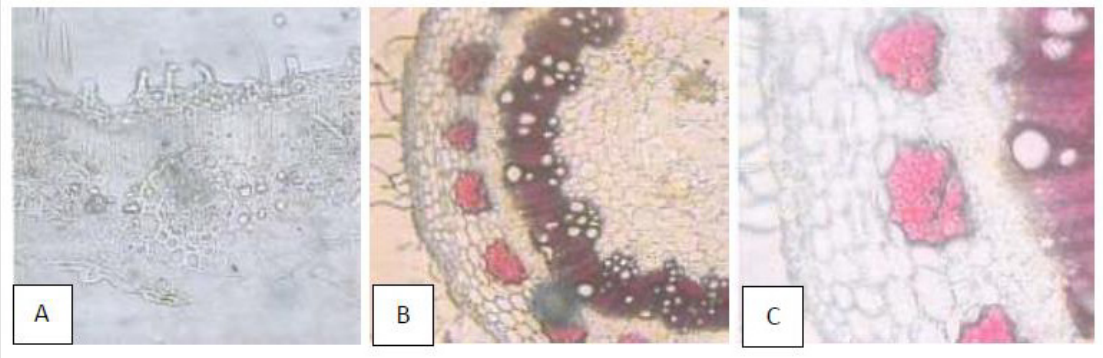

Figure 2:

a) Leaf: Detailed transverse section at the midrib region covering and glandular trichomes on the upper epidermis, palisade (one layer) spongy tissues, vascular tissues, lower epidermis (less covering trichomes) coloured particles (in palisade and spongy parenchyma cells). Vascular elements are surrounded by characteristic cells with thick cell walls and wide lumen.

b) From outside to inside (TS of stem): Covering trichomes, epidermal layer (small globular cells covered with a thick cuticle), several layers of cortical collenchyma, groups of lignified fibres of the cortex (purple in colour), phloem tissues, heavily lignified xylem tissues (vessels, tracheids, fibres (dark violet) and at the centre the parenchyma cells of the pith.

c) From outside to inside (TS of stem): Epidermal layer (small ovoid cells with thick cell walls) bearing covering trichomes, cortical collenchyma with thick cells, groups of lignified fibres with thick cell walls and narrow lumens, phloem tissues, vessels, tracheids and xylem fibres (heavily lignified).

Chemical Constituents

Alkaloids, fixed and volatile oils, saponins diasogenin, ruscogenin, resin and sapogenin [2]. Compounds: terrestribisamide, 25R-spirost-4-en-3,12-dione and tribulusterine. N-p-coumaroyltyramine, terrestriamide, hecogenin, aurantiamide acetate, xanthosine, fatty acid ester, ferulic acid, vanillin, p-hydroxybenzoic acid and β-sitosterol in dried fruits [3]. The steroidal saponins: protodioscin, prototribestin, pseudoprotodioscin, dioscin, tribestin and tribulosin. The flavonoid rutin. Furostanol saponin (tribol), and sitosterol glucoside [4]. furostanol saponins from the fruits : terrestroside A [5]. Furostanol glycosides: tribufurosides I and J from the fruits [6]. sitosterol-D-glucoside and a spirostanol type steroidal saponins: Tribulosin [7]. also contains the steroidal saponin: protodioscin [8]. Steroidal saponins in the aerial parts : Protodioscin, neoprotodioscin and prototribestin [9]. Aerial parts: flavonoids, tannins,sterols and/or triterpens and volatile oils. leaves and fruits: flavonoids kampferol, kamferl 3-glucoside, kaempferol 3-rutino-side and tribloside.

Steroidal saponins diosgenin. Chlorogenin and gitogenin, ruscogenin, hecogenin, neotigogenin, and 3-deoxy Δ^3-diosgenin, β- sitosterol and stigmasterol. Steroid glycoside dioscin, trillin, diosgenin-Dglycoside and gracillin in addition to Harman and harmine alkaloids from the aerial parts. The flowers contain sterols (stigmasterol, campesterol and β-sitosterol), sapogenins (diosgenin, gitogenin, neogitogenin), flavonoidal aglycons (Kaempferol, quercetin) and reducing sugars (D-glucose, D-arabinose and L-rhamnose).The aerial parts contain steroidal saponins main furostanol bisglycosides [10].

Physicochemical Constituents (%)

Physico chemical constituents carried out on the plant *Tribuls terrestris* are as follows:

- **Loss of weight in drying at 105°C : 7.09**

Absolute alcohol solubility	: 3.20
Water solubility	: 21.20

- **Successive extractives (%)**

Petroleum ether (60-80°C)	: 1.75
Chloroform	: 1.55
Absolute alcohol	**: 5.70**
Distilled water	: 18.00

➢ **Ash values (%)**

Total ash	: 10.60
Water soluble ash	: 3.60
Acid insoluble ash (10% HCl)	: 1.30

➢ **pH values (aqueous solution)**

pH of 1% solution	: 5.98-6.05
pH of 10% solution	: 5.47-5.48

Elemental Analyses

(Table 1).

Table 1.

Ash values (British Herbal Pharmacopeia- Reference)					
Assay and identification of element (AOAC International -Reference)					
Apparatus	(AA-6800 Shimadzu-Flame method)				
Element	Std. conc. µg/ml(ppm)	Sample conc.mg/ml	Sample absorbance	Actual conc.mg/ml	Actual conc. (%)
Cr	1, 2, 4	10	0.0005	0.00081	0.000081
Zn	0.25, 0.5, 1	10	0.1489	0.04472	0.004472
Cu	1, 2, 4	10	0.0148	0.0178	0.00178
Fe	1, 2, 4	0.909	0.2053	0.38748	0.038748
K	1, 2, 4	0.909	1.0576	8.6685	0.86685
Pb	1, 2, 4	10	0.0000	<0.01 µg/g	0.0000
Cd	0.125,0.25,0.5	10	0.0000	<0.001 µg/g	0.0000
Ca	5, 10, 20	0.0826	0.1130	26.937	2.6937
Mg	0.25, 0.5, 1	0.0826	1.0394	10.7003	1.07003
Na	1,2,4	0.909	0.1242	2.84108	0.284108

UV Spectral Studies

(Table 2, Figures 3 & 4).

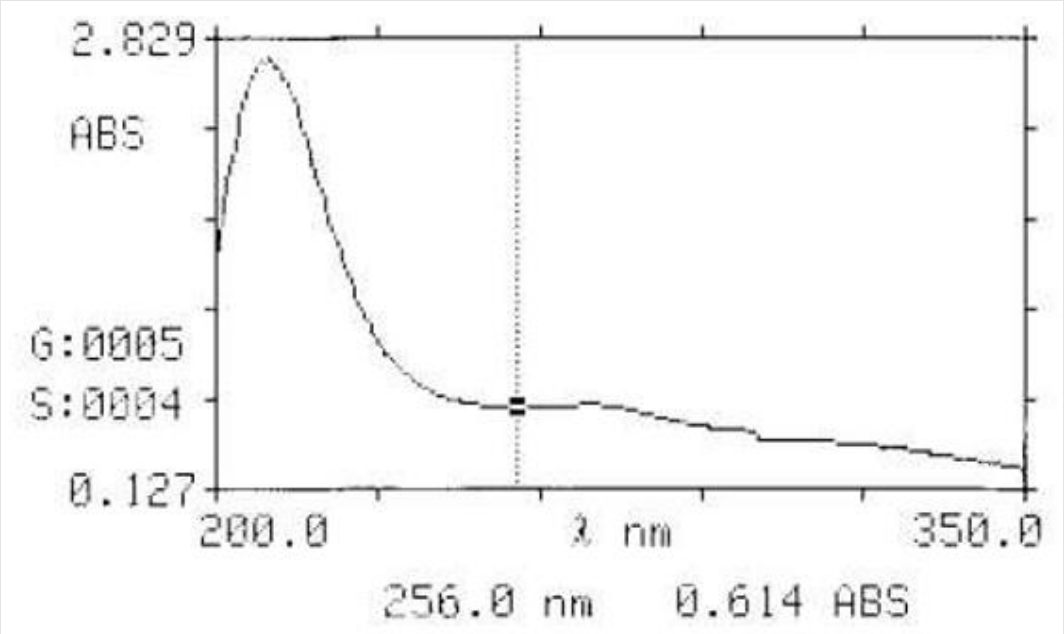

Figure 3: Intestinal Fluid simulated without pancreatic pH=7.5±0.1.

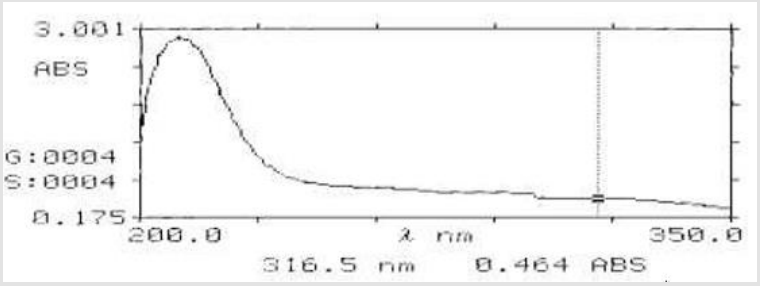

Figure 4: Gastric Fluid simulated without pepsin pH=1.2±0.1.

Table 2.

Ultraviolet Spectrum (USP reference)				
Apparatus	**Beckman DU 520 general purpose UV/VIS spectrophotometer**			
Sample conc. (mg / ml)	**Solvent**	**λ max (nm)**	**λ min (nm)**	**Abs.(λ max - λ min)**
0.529	Intestinal Fluid simulated without	210.5		2.693
	pancreatic pH=7.5±0.1	269	256	0.635-0.614
0.875	Gastric Fluid simulated without	316.5	312.5	0.464-0.466
	pepsin pH =1.2±0.1	313	310	0.467-0.466
		309.5	305.5	0.467-0.466
		307.5	304	0.467-0.466
		304.5	301.5	0.468
		302	284	0.469-0.469
		286.5	282	0.551-0.550
		283.5	279	0.551-0.551
		281		0.551-0.550
		210		2.87

Thin layer Chromatography (TLC)

Figure 5.

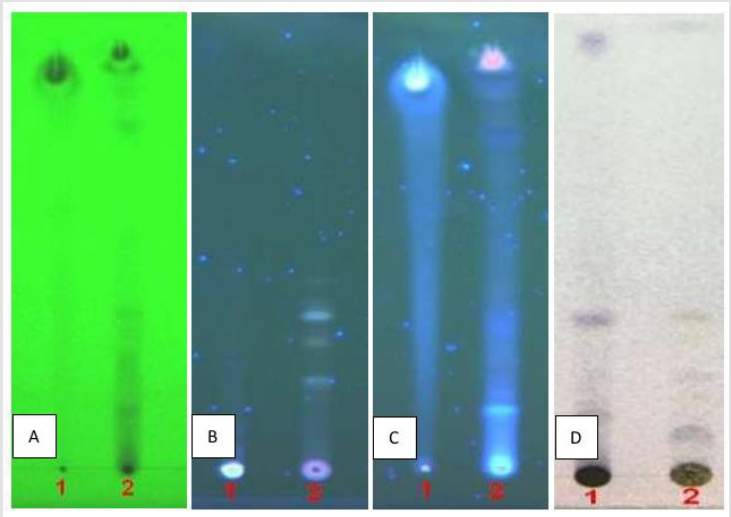

Figure 5: TLC fingerprint of Petroleum ether -60-800 (track 1) and MeOH extract (track 2) Mobile phase.
- A&C: Ethyl acetate, methanol, water (100:13.5:10).
- B&D: Toluene, ethyl acetate (93:7)
- Detection A: UV 254nm
- B&C: UV366nm
- Derivatization D: Vanillin- Sulphuric acid-vis.

Pharmacological and Toxicological Studies

Information reported in the Literature about the plant: The important pharmacological and toxicological activities of the plant *Tribulus terrestris* reported in various scientific journals have been presented in the present brief review: A mixture of 9 oriental herbs were given including *Tribulus terrestris* showed an increase in the copulatory behavior parameters in rats, improved the sexual activity and erectile function. The effects of methanolic and aqueous extracts of *Tribulus terrestris* on rat blood pressure (BP) and the perfusion of mesenteric vascular bed were investigated and found possessing significant antihypertensive activity in spontaneously hypertensive rats [11]. Effect of Saponins from *Tribulus terrestris* (STT) on the renal carcinoma cell (786-0) *in vitro* and inhibitory mechanisms studied significantly inhibited the growth of 786-0 *in vitro*, partially, by apoptosis [12]. *Tribulus* is a genus of plants found in many warm regions.

The best-known member is *T. terrestris* (Puncture Vine), a widespread weed and also the source of a dietary supplement claimed to increase the body's natural testosterone levels and thereby improve male sexual performance and help build muscle. *T. terrestris* has consistently failed to increase testosterone levels in controlled studies [13-15]. It has also failed to demonstrate strength-enhancing properties [16]. *Tribulus* has been shown to enhance sexual behaviour in an animal model. It appears to do so by stimulating androgen receptors in the brain [17]. Jameel, et al. [18] reported a case of a young weight-trainer who developed gynaecomastia due to oral intake of an herbal tablet which he used as a steroid alternative for body-building. The aqueous extract of *Tribulus terrestris* can significantly increase melanocyte-stimulating hormone (MSH) expression in the hair follicle melanocytes by activating tyrosinase activity and promoting melanocyte proliferation, melanine synthesis, and epidermal migration of dormant melanocytes [19]. *Tribulus terrestris* showed protective effect for STZ-induced diabetic rats may be mediated by inhibiting oxidative stress [20].

Sharif, et al. [21] investigated the antihypertensive mechanism of *Tribulus* in 2K1C hypertensive rats by measurement of circulatory and local ACE activity in aorta, heart, kidney and lung. The ACE activity in all tissues of *Tribulus* fed hypertensive rats was significantly lower than that of hypertensive rats, which was more pronounced in kidney. These results indicated that there is a negative correlation between consumption of *Tribulus* and ACE activity in serum and different tissues in 2K1C rats. Oludotun, et al. [22] reported the effects of methanolic and aqueous extracts of *Tribulus terrestris* on rat blood pressure (BP) and the perfused mesenteric vascular bed were investigated. The extracts dose-dependently reduced BP in spontaneously hypertensive rats (SHRs) with the aqueous fraction being more potent than the methanolic fraction at all doses tested. *In vitro*, the methanolic but not aqueous extract produced a dose-dependent increase in perfusion pressure of the mesenteric vascular bed. When perfusion pressure was raised with phenylephrine, the aqueous extract produced a dose-dependent reduction in perfusion. It was concluded that methanolic and aqueous extracts of *Tribulus terrestris* possess significant antihypertensive activity in spontaneously hypertensive rats.

The antihypertensive effects appeared to result from a direct arterial smooth muscle relaxation possibly involving nitric oxide release and membrane hyperpolarization. The inhibitory effect of saponins from *Tribulus terrestris* (STT) on Bcap-37 breast cancer cell line were determined by cell growth curve, MTT assay, protein content assay and morphological observation showed that STT had potent inhibitory effect on Bcap-37 cell line in a concentration-dependent manner [23]. The results of pharmacological and Toxicological studies carried on the 70% alcoholic extract of the plant, have been given below (Figures 6-8 and Table 3).

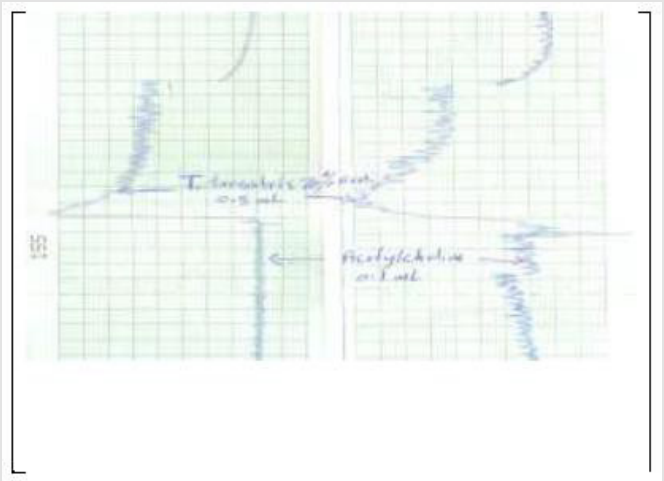

Figure 6: Effect of Tribulus t. on rat detrusor muscle acetylcholine treated.

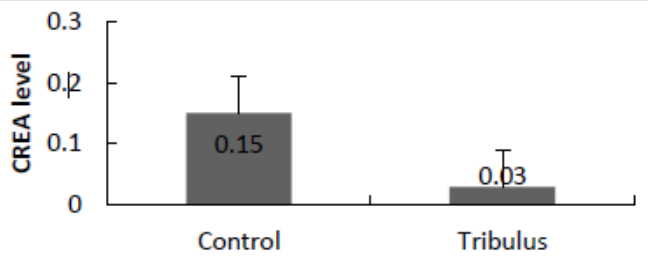

Figure 7: Effect of Tibulus 70% ethanol extract on serum createnin level.

Table 3.

Activity	Results			
	Strong	Moderate	Mild	Negative
Anti-inflammatory (Ear edema)				√
Anti-diabetic activity (STZ)			√	
Antidepressant			√	
Anticonvulsant				√
Effect on mesenteric artery		√		
Endocrinological studied				√
Effect on Guinea pig ileum		√		
Rat detrusor muscle		√		
Effect on corpus cavernous strip			√	
Antithrombotic activity				√
Acute toxicity on mice				√
Biochemical studies				√
Hematological studies (MCH & PLT)		√		
Effect on kidneys weight		√		
Locomotor activity test				√
Motor co-ordination (Rota rod test)				√
Rectal temperature				√
Body weight				√
Mortality				√
LD50 = > 10g/kg p.o. in mice				

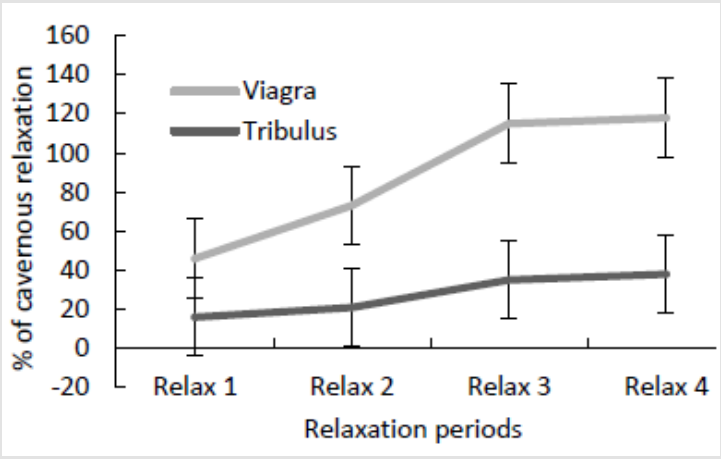

Figure 8: Tribulus Sexual study followed by Viagra.

Conclusion

The oral administration of the plant extract was non-toxic up to the dose of 10 g/kg b.wt., p.o. These acute studies demonstrated that the plant extract is safe and did not cause any detrimental effects *in vivo* under the conditions investigated in this study. The LD50 of plant extract was found to be >10 g/kg when administered once via gastric intubation to rats. Plant extract produced a mild relaxation in phenylephrine pre-contracted corpus cavernous tissue.

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