GOKHRU *(TRIBULUS TERRESTRIS* L.): A TRADITIONALLY IMPORTANT WILD MEDICINAL HERB OF WASTE LANDS

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INTRODUCTION

Gokhru (*Tribulus terrestris* L.) Family, Zygophyllaceae, vernacular name Gokhru, Bhakra, Nebula, Puncture vine, Goat head, Mexican sandbur and Texas sandbur, is a prostate branched annual or biennial herb with 0.6-1.5 long trailing hirsute or silky stem. Leaves are opposite and paripinnate with 4-8 parts of small silky, oblong leaflets. Flowers are borne solitary or in short auxiliary peduncles, with five yellow petals.

Fruits are five angled with two long and small spines, coarsely hairy and contain 2-5 seeds. The species is widespread in mediterranean subtropical and desert climates worldwide. It grows rapidly along roads and waste places. In India, it thrives well in sandy or black irrigated soil upto altitude of 3000m. It is native of Europe and Asia and puncturevine may have been introduced to the United States and other sources from the Mediterranean region.

The major biochemical constituents of *Tribulus terrestris* are steroidal saponins like protodioscin, ruscogenin, hecogenin and diosgenin, many alkaloids, phytosterols, namely beta-sitosterol, stigmasterol, cinnamic amide derivatives and many flavonoids such as rutin, kampferol, and quercetin. Various tannins, fatty acids, calcium, magnesium, potassium, and iron components are also found in *Tribulus terrestris* (Duhan, 1992).

Tribulus terrestris plants have been widely used in the various systems of medicine throughout the world for a variety of diseases. It is also used as mood enhancer and for building muscles and stamina in sports persons.

Different aspects of *Tribulus terrestris* study

1. Morphological field survey and nodulation study on *Tribulus terrestris*:

Japanese worker (Y Fukuda 1982) carried out the morphological and anatomical studies in the seedlings of *Tribulus terrestris*. The seedlings possess four buds immediately above the cotyledonary lobe that grow into prostate shoots. All the leaves including cotyledonary are vascularied by four vascular bundles among which two are related to a single median gap. Athar and (Mahmood, 1985) observed the Root nodulation in *Tribulus terrestris* growing in the field under natural growth conditions has been studied. Nodules occurred singly as well as in branched forms and averaged 1.5-2.0 mm in diameter and maximum nodulation was observed in sandy soil and sandy gravels. The enzymatic reduction of tetrazolium salt by *Tribulus terrestris* nodules rendered evidence for the presence of intense dehydrogenase activity, thus indicating a strong possibility of nitrogen fixation in the nodules. (Ernst and Tolsma, 1985) studied that Dormancy in the seeds of *Tribulus terrestris* can last for 3-6 years, resulting in a very irregular pattern of germination. (Boydston, 1980) investigated that puncture vine flowered within 3-4 weeks after emergence when temperatures were favorable and produced an average of 5600, 5200, 3600 and 200 burs per plant planted in May, June, July and August respectively.

Flowers and fruiting occur from July to October, depending upon geographical location. Plant reproduces by seeds which germinate soon after first rain in spring and summer; seeds may remain viable for 4-5 years. Dispersal of spiny burs is with attachment to animals, humans or the tires of (Vehicles, 1996). Scott and Morrison, (1996) identified the origins of the widespread weed and potential biological control of *Tribulus terrestris*. Measurement were made of four size variables, four spine angles and the number of seeds in each burr from 31 Australian and overseas collection sites. The relationship between base length and width of the burr distinguished the probably native northern Australian collections, which have either more square or more elongate burrs, from the probably introduced populations of *Tribulus terrestris* in southern Australia. A trial key note is represented to some species of *Tribulus* L. which occur in Australia. In contrast, the taxonomy of those species surrounding the *Tribulus terrestris* complex is

solely in need of a world revision two taxa are informally described with *Tribulus terrestris* complex **(**Barker, 1998).

GOKHRU

(A) Whole plant

(B) Flower

(C) Fruit

2. Tissue culture and plant regeneration study of *Tribulus terrestris:*

Erhun and Sofowora (1986) studied the callus culture induced from the leaf and stem portions of *Tribulus terrestris* were shown to contain steroidal sapogenins. Auxins (2, 4-D & NAA) and cytokinins (N6BA & kinetin) were used on callus induction. A combination of 2.5 times 10^{-6} M 2-4-D & 2.0 times 10^{-6} M kinetin was found to be best for callus induction and maintenance.

Direct regeneration of shoots and roots has been achieved in *Tribulus terrestris* cotyledonary leaves along with epicotyls segment from young seedlings. These were cultured on MS medium containing various concentrations of auxin with cytokinin and glutamine. Morphogenic responses such as present shoot and root differentiation were recorded as regular intervals (Ali, 1997).

Mohan and associates (2000) studied the somatic embryogenesis and plant regeneration from the stem explants of *Tribulus terrestris*. Murashige and skoog,s medium supplemented with 0.5 mg 1-1 2, 4, -dichlorophenoxyacetic acid +.5mg 1-1 kinetin was best for callus induction and callus maintenance.

3. Phytosociological, ecological and nutritious food related survey on *Tribulus terrestris:*

The quantitative analysis of food of Houbara bustard (*Chlamydotis undulate macqueeni*) revealed that a total 23.30% of its food is derived from *Tribulus terrestris* (Main, 1986). Duhan and coworkers (1992) surveyed the non-conventional foods including fruits, leaves and grains consumed in various parts of Indian sub continent for their nutritional value. *Tribulus terrestris* was found to be rich source of calcium, phosphorus and iron. In a survey on the two Hausa villages for food procurement practices, with special focus on dietary use of wild plant it was found that more than 80 species of plants including *Tribulus terrestris* were consumed (Humphry, 1993). Because protein value of *Tribulus terrestris* plants were exceeding 20%. Studies carried on the diet and plant species preferences of two sympatric tortoises in South Africa and found that *Tribulus terrestris* featured in diets during different periods and consumption pattern show the climatic affinities of the two species (Rall, 1993). *Tribulus terrestris* plant extract could be one of the cheaper and abundant source of insect's hormones and can be used to increase the silk yield in commercial silkworm rearing (Rajashekhargouda, 1997). *Tribulus terrestris* exert adversely affects on the growth of other herbs in its vicinity. Preliminary analysis indicated the presence of phenolic compounds in the leachate of *Tribulus terrestris* which are believed to play a significant role in growth inhibition of other annual plants. (EL Gareeb, 1991).

4. Cytogenetics and Breeding behaviour:

The phenomenon of double fertilization that starts with pollen tube opening and ends with sperm merging into female nuclei has been demonstrated in *Tribulus terrestris* by Balyaeva (1980). The breeding behaviour and pollination ecology of the *Tribulus terrestris* L. populations growing at Visakhapatnam India have been studied by Reddi and associates (1981). The plant shows autogamy with perfect seed setting. Stamens bend inwards and gain contact with at 1800 h. However a variety of insects visits the flowers for pollen and stigma or nector during 0600-1500 h and brings about pollination as well. The author highlighted the role of the species in providing sustenance to these insects to remain in the ecosystem until required by sympatric species for pollination sources. Heiser and coworkers (1948) were probably the first to report the chromosome number 2n=24 for T. terrestris Porter (1968) suggested this species to be based on x=6. A higher polyploidy (8x) 2n=48 was reported by Fotedar (1969). Rashid (1974) reported 2n=36 for mediterranean

region. C P Malik (1966b) carried out investigations on the Indian population of species and reported the occurrence of intraspecific cytotypes with 2n=12, 24, 36 & 48. Basic chromosome number (n=6) in *Tribulus terrestris* was reported from Rajasthan desert of India. There is no relationship between morphological diversity and variation in chromosome number and it is suggested that morphology should not be used in discriminating in determining the ploidy nature of an organism (Bhansali 1990). Mesicek and Sojak (1995) found the hexaploid chromosome numbers 2n=36 in Mongolian *Tribulus terrestris*. Morrison and Scott (1996) detected the three ploidy levels tetraploid (2n=24), hexaploid (2n=36), and octaploid (2n=48) in *Tribulus terrestris* from 24 Australian and 24 overseas collections. This chromosome number variation includes the identification and origin of this widespread weed. Which provide further evidence for a base number of x=6 for the genus. They also investigated isozyme variation in seedlings as part of a study to identify the origins of the widespread weed *Tribulus terrestris* L. polymorphism was detected in 8 of the 11 putative loci scored (1996)**.**

5. Phytochemical analyses

It includes determination and identification of different biocomponents using various techniques. The distribution and metabolism of gamma-methyl ene-glutamic acid, other amino acids and amides during the fruit growth in *Tribulus terrestris* was studied and it was found that the amount of .gamma.-methyleneglutamic acid dominated over gamma.-methyleneglutamine (Jain, 1981). The free endogenous ascorbic acid content in the roots of *Tribulus terrestris* was (40.0 + - 1.30 mg/100g.d.w) (Nag, 1986). Saleh and others (1982) detected the 25 flavonoid glycosides from *Tribulus terrestris*. These glycosides belong to the common flavonoids-kaempferol, quercetin and isorhamnetin, with the 3-gentiobiosides as the major glycosides. Louveaux and coworkers (1998) detected 18 flavonoids (caffeoyl derivatives, quercetin glycosides including rutin and kaempferol glycosides) with HPLC in the leaves extracts of *Tribulus terrestris*. The chemical and pharmacological screening of Saudi Arabian *Tribulus terrestris* revealed the presence or absence of alkaloids, cardiac glycosides, tannins, volatile oils, volatile bases and glucosinolates (Mossa, 1983).

Huang, Zhang and Liang (1991) extracted the crude polysaccharides from the stem and leaf of *Tribulus terrestris* L. A homologous polysaccharide H obtained by gradation and purification contains Ara, Rha, Xyl, Gala. Gal & Glc. in molar ratios of 1.6:2.4:0.1:3.5:1.3:1. Isolated two cinnamic amide derivatives named terresttriamide (1) and a known compound, 7-methylhydroindanone from *Tribulus terrestris* L. for the first time by Ren (1994). Mahato and others (1994) isolated the beta-sitosterol-beta-D-glucoside and dioscin, (Both new steroidal glycosides), neohecogenin glycoside and tribulosin from the aerial part of *Tribulus terrestris*. Further studies on the constituents of the fruits of *Tribulus terrestris* lead to the isolation of five new steroidal saponins and their structure were elucidated through 2-D NMR techniques (Yan 1996). Two steroidal saponins named 3-0-beta-glucopyranosyl (1 fwdarw 4) –beta-D-galactopyranoside from the aerial parts of *Tribulus terrestris* were isolated on the basis of chemical and spectroscopic evidence (Wu G 1996). Wilkins and associates (1996) found that GC-MS analysis of the hydrolyzed ethanol-water (4:1) extracts of *Tribulus terrestris* specimens from two of four sites, revealed high levels of ruscogenin and potentially lithogenic diosgenin saponins.

In 1997 isolated six new furostanol saponins from the fruits of *Tribulus terrestris* and their structures were elucidated on the basis of spectroscopic techniques (Wang 1997). Fang (1998) extracted two major steroidal saponins from *Tribulus terrestris* and considerable useful structural information was obtained. Steroidal saponins, (5 alpha, 25 R)-spirostan-3, 6, 12-trione and 25 R-spirostan-4-nee-3, 6, 12-trione, together with 5 known steroidal sapogenins were isolated from puncturevine and also established their structures through 2D NMR spectroscopic techniques(Xu 1998). Three new compounds, terrestribisamide, 25R-spirost-4-en-3, 12-dione and tribulusterine, together with 10 known compounds from dried fruits of *Tribulus terrestris* by Wu and coworkers (1999) and their structures were determined by spectral analysis. Three new steroidal saponins, together with 5 known saponins, L. mannitol and an organic salt in *Tribulus terrestris* L. and structures of these compounds were elucidated by 1D & 2D NMR spectra (13C-1H COSY, HMQC,HMBC,1H-1H COSY, TOCSY & NOESY) mass spectrometry (FABMS, ESIMS) and chemical methods (47). Xu (2001) identified two new compounds as neohecogenin-3-O-beta-D-glucopyranosyl (1 fwdarw 2)-beta-D-glucopyranosyl (1 fwdarw 4)-beta-D-galactopyranoside (I); neohecogenin-3-O-beta-D-glucopyranosyl (1 fwdarw 4)-beta-D-galactopyranoside (II).

Kostova (2002) reported the known furostanol saponins methylprotodioscin and protodioscin and two new sulfated saponins, sodium salt of 26-O-beta-glucopyranosyl-22 alpha-methoxy-(25R)-furost-5-ene-3beta,26-diol-3-O alpha-rhamnopyranosyl-(1fwdarw2)-beta-4-O-sulfo glucopyranoside (methylprototribestin) and sodium salt of 26-O-beta-glucopyranosyl-22alpha-hydroxy-(25R)-furost-5-ene-3beta,26-diol-3-O-alpha-rhamnopyranosyl-(1fwdarw 2)-beta-4-O-sulfo-glucopyranoside (prototribestin) from the aerial parts of *Tribulus terrestris* L. growing in Bulgaria. Ganzara and his team (2001) described the first analytical method suitable for the determination of steroidal saponins in *Tribulus terrestris* by using a reversed-phase (RP-18) column, evaporative light scattering (ELS) detection interestingly, there exists

considerable variations of 0.17 to 6.49% in the protodioscin content depending on origin and plant part used for extraction.

De-Combarieu and others (2003) has developed an HPLC-ELSD-ESI-MS method for the analysis of the steroidal saponins (Protodioscin: 5, 6-dihydroprotodioscin, neoprotodioscin, and their respective sulfates) in the aerial parts of *Tribulus terrestris*. Pilipenko and Sukhodub (2003) applied 252Cf time-of-flight plasma desorption mass-spectrometry (TOF-PDMS) for investigation of hecogenine and diosgenine steroid glycosides from *Tribulus terrestris* L. The phytochemical investigation of the aerial parts of the plant of Bulgarian origin studies the resulted in the isolation of the novel furostanol saponin 1, named tribol, together with the known spirostanol saponins 2 and 3 and sitosterol glucoside (Conrad, 2004). A sensitive, simple, and accurate reversed-phase high-performance thin-layer chromatographic method (Sane 2004) for determination of protodioscin in fruit powder from *Tribulus terrestris* L was developed.

6. Pharmacological and clinical investigation on puncture vine:

Tribulus terrestris has been used in many medicinal systems of the world for its wide range of medicinal properties. Test animals studied for action of *Tribulus terrestris* plant extract are mice, guinea pig, frogs and rabbits etc (Mossa, 1983). The researches conducted on the plant have revealed useful pharmacological properties of the plant. Kemertlize (1982) prepared a new antisclerotic drug from *Tribulus terrestris* which was recommended for clinical use in prevention of atherosclerosis. It was found that the extracts of *Tribulus terrestris* exhibited significant analgesic activity versus benzoquinone-induced writing and in thermal tests (Twaij, 1987). The plant (TT) caused inhibition of adrenaline-induced aggregation of human platelets (Sajid, 1991). Sharifi (2003) investigated the antihypertensive mechanism of Tribulus in 2K1C hypertensive rats by measurement of circulatory and local ACE activity in aorta, heart, kidney and lung and found that 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. The inhibitory and apoptosis-inducing effects of saponins from Tribulus terrestris (STT) on liver cancer cell line BEL-7402 were studied and found that saponins from *Tribulus terrestris* exerts its cytotoxic effect on BEI-7402 cells by inducing apoptosis (Sun ,2004).

Studies conducted on animals and man by different groups of workers confirmed that TT has potentials to improve various aspects of sexuality. The plant extracts improved the libido and sexual activity especially in pigs with reduced sexual effects (Koumanov, 1982). Similar effects have been found in hypogonadal men and that was improved in seminological induces in infertile men (Protich, 1983). In another clinical study TT was found to be effective against idiopathic oligo-ashheno-terato-zoospermia (Moeloek, 1994). Zarkova, (1984) studied the stimulatory effect on the sexual functions in rats due to Tribestan (obtained from *T. terrestris*). Adimoelja (2000) isolated the phytochemical agent (protodioscin) from *Tribulus terrestris*, which has been clinically proven to improve sexual desire and enhance erection via the conversion of protodioscine to DHEA (De-Hydro-Epi-Androsterone). Adaikan and others (2000) investigated the effects of oral treatment of *Tribulus terrestris* extract on the isolated corpus cavernosal tissue of New Zealand white rabbits and to determine the mechanism by which protodioscin (PTN), a constituent of the *Tribulus terrestris*, exerts its pharmacological effects. *Tribulus terrestris* extract appears to possess aphrodisiac activity probably due to androgen increasing property by protodioscin (as aphrodisiac) Gauthaman (2002). PTN is known to increase the quality of semen in terms of motility and quantity Stanislavov and Nikolova (1999) increase level of testosterone, leuteinizing hormone and dehydroepiandrosterone Koumanov (1982).

The plant is represented to have toxic properties as given below-

Shoots and fruits of *Tribulus terrestris* have medicinal value for its antimicrobial activity against Staphylococcus aureus and *Escherichia coli* (71). Ethanolic extract of *Tribulus terrestris* showed antibacterial activity against both Gram-positive and Gram-negative bacteria and has cytotoxic effects also (72). Twaij (73) presented a study in which molluscidal activity was shown by the plant *Tribulus terrestris* with lethal concentration of 50-100 ppm against Bulinus truncatus. Singh (74) found that essential oils from the *Tribulus terrestris* (seeds) screened against the larva of root knot nematode meloidogyne incognita (kofoid &white) chit wood was found to be very effective. The extract of Tribulus terrestris with 50% methanol has anthelmintic activity in-vitro using the nematode caenorhabditis elegans (75). Ali (72) reported *Tribulus terrestris* show cytotoxic, however Liu (76) indicated that *Tribulus terrestris* polysaccharides have no mutagenic effect on mice, but protect chromosome and DNA damages. Tribulusamides A and B, new lignanamides embracing two cinnamic amide, from the fruits of *Tribulus terrestris* (Surender, 1986). Addition of these compounds to primary cultured mouse hepatocytes significantly prevented cell death induced by D-galactosamine (D-GalN)/tumor necrosis factor alpha (TNF-alpha).

A preliminary study revealed that the water extracts of *Tribulus terrestris* has diuretic effects in albino rats, which

was attributed presence of high CMC of potassium (Kumari, 1967). In similar studies diuretic action with minimal side effects was confirmed (Singh, 1991).

Singh (1990) investigated on the influence of indigenous diuretics *Tribulus terrestris* (Gokshura) on the pattern of serum and urinary electrolysis in an experimental model using albino rats. An ethanolic extract of the fruits of *Tribulus terrestris* has significant dose dependent protection against urolithasis induced by glass bead implantation in albino rats (Anand 1994). Further study revealed that aqueous extract of *Tribulus terrestris* has urinary inhibitory activity towards calcium oxalate crystal growth in rats fed sodium glycolate (Sangeeta 1994). Ali (2003) found that diuretic and contractile effects of *Tribulus terrestris* indicate potential of propelling urinary stones and merits further pharmacological studies.

Tribulus terrestris effects cardiovascular system low doses of *Tribulus terrestris* extracts increase heart rate while higher doses seen to decrease in frog (Chakraborty, 1978) and guinea pigs, (Seth, 1976) heart. *Tribulus terrestris* improved the coronary perfusion with no adverse effects on long term use (Wang, 1990).

Recently the effect of *Tribulus terrestris* on nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) activity and androgen receptor (AR) immunoreactivity in rat brain was studied and found that chronic treatment of *Tribulus terrestris* in rats increases the NADPH-d positive neurons and AR immunoreactivity in the PVN region (Gauthaman, 2005). The observed increase in AR and NADPH-d positive neurons in the present study is probably due to the androgen increasing property of *Tribulus terrestris*.

CONCLUSION

Protodioscin and quercetin are two value aided products of *Tribulus terrestris* with immense future. There exists a wide spread variation in the genotypes of the plants growing in diverse locations of varied environments. And there is no correlation of chemical analysis data with geographical distribution and polyploidy of *Tribulus terrestris*. There is ample need to work for genetic and biochemical characterization to improve the quality and quantity of discussed valued products for pharmaceutical formulation development.

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