ANTIOXIDANT ACTIVITY OF AERIAL PARTS OF *TRIBULUS ALATUS* IN RATS

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ABSTRACT

The antioxidant activity of alcoholic extract of *Tribulus alatus* was investigated by determination of blood glutathione, serum ascorbic acid and serum superoxide dismutase in rats. All groups treated with aerial parts without fruit, fruits and total herb showed a significant increase in all measured parameters ($P<0.05$). Upon fractionation of the alcoholic extracts using solvents with different polarities, all fractions revealed a significant increase in serum superoxide dismutase ($P<0.05$). On the other hand chloroformic fraction of aerial parts without fruit extract and ethylacetate fraction of fruits extract exhibited a significant increase in blood glutathione level. All fractions of fruits extract, chloroformic and ethylacetate fractions of aerial parts without fruit extract significantly increase the serum ascorbic acid concentration ($P<0.05$).

Keywords: *Tribulus alatus*, glutathione, ascorbic acid, superoxide dismutase.

INTRODUCTION

The genus *Tribulus* of the Zygophyllaceae comprises ca 20 species which grow as shrubs or herbs in subtropical areas around the world (Pope 1968, Aplin 1976). Among the *Tribulus* species *T.terrestris* and *T.cistoides* had been phytochemically investigated (Tamova *et al*., 1965, Tamova *et al*., 1978, Mahato *et al*., 1981, Achenbach *et al*., 1994, Achenbach *et al*., 1996).

On the other hand, *T.terrestris* is used in folk medicine against various diseases (Chakraborty *et al*., 1978). *Tribulus alatus* is an annual or biennial prostrate herb, found in dry sandy soil along roads in warm-temperate regions. Its fruits are used in Pakistan for the treatment of urinary disorders and cough (Täckholm 1974 and Ghazanfar 1994).

In previous study the isolation and characterization of eight steroidal saponins, six of them are new steroidal glycosides based on spirostane , furostane and cholestane aglycone, together with six flavonol glycosides based on Kaempferol, Isorhamnetin and Quercetin aglycone, have been reported from *Tribulus alatus* growing in Egypt (Temraz *et al*., 2006).The aim of this work was to investigate the antioxidant activity of alcoholic extract of aerial parts and their fractions of *Tribulus alatus*.

MATERIAL AND METHODS

Plant material and extraction

Samples of *Tribulus alatus* were collected from Al Azahar University, Nasr city, Cairo and were kindly identified by Dr. Nabil El-Hadidy, Professor of Botany, Faculty of science, Cairo University. A voucher specimen was deposited at Pharmacognosy department, Faculty of Pharmacy, Al Azhar University.

The plant was divided into two parts. Both parts were studied separately and the antioxidant activity was compared to that of the total plant.

Part 1: aerial part without fruits.
Part 2: fruits.
Both parts of the plant (2 kg, 0.4 Kg respectively) were finely powdered and were macerated in 70% methanol. The alcoholic extract was evaporated to dryness under vacuum. The process of maceration and evaporation was repeated till exhaustion of the plant powder.

The residues for both parts were combined, weighed (434 g) (120 g), suspended in distilled water and successively extracted with chloroform, ethylacetate and *n*-butanol saturated with water. Each extract was collected and evaporated to dryness under vacuum to give chloroformic extract (15 g), (8 g) ethylacetate extract (8 g), (6.2 g) and butanolic extract (34 g), (11.8 g), then the water fraction left after fractionation was evaporated to dryness and the residue was macerated in absolute ethanol several times. The alcoholic extracts were combined and evaporated to dryness under vacuum to give ethanolic extract (150 g), (32 g) respectively.

Animals

A total of 72 adult albino male Wistar rats weighing 160-180 g were used in this experiment. The animals were kept under standard conditions and were classified as

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follow:

Group 1: Received 50 mg/Kg body wt. of 70 % alcoholic extract of part 1.
Group 2: Received 50 mg/Kg body wt. of 70 % alcoholic extract of part 2.
Group 3: Received 50 mg/Kg body wt. of 70 % alcoholic extract of total herb.
Group 4: Served as control group.
Group 5: Received 12.5 mg/Kg body wt. of chloroformic fraction of the alcoholic extract of part 1.
Group 6: Received 12.5 mg/Kg body wt. of ethylacetate fraction of the alcoholic extract of part 1.
Group 7: Received 12.5 mg/Kg body wt. of butanolic fraction of the alcoholic extract of part 1.
Group 8: Received 12.5 mg/Kg body wt. of ethanolic fraction of the alcoholic extract of part 1.
Group 9: Received 12.5 mg/Kg body wt. of chloroformic fraction of the alcoholic extract of part 2.
Group 10: Received 12.5 mg/Kg body wt. of ethylacetate fraction of the alcoholic extract of part 2.
Group 11: Received 12.5 mg/Kg body wt. of butanolic fraction of the alcoholic extract of part 2.
Group 12: Received 12.5 mg/Kg body wt. of ethanolic fraction of the alcoholic extract of part 2.

The extracts were suspended in distilled water using Tween 20, and the dose was orally administered once daily for 6 weeks. At the end of treatment, blood samples were collected, centrifuged and serum was separated for the determination of the following:

1- Blood glutathione content according to the method described by Beutler *et al*. (1963).
2- Serum superoxide dismutase activity, the method was carried out according to the pyrogallol method of Marklund *et al*. (1974).
3- Serum ascorbic acid was estimated by the method of Jagota *et al*. (1982).

RESULTS AND DISCUSSION

Different groups treated with extracts of aerial parts without fruits (part 1), extract of fruits (part 2) and total herb extract showed a significant increase in blood glutathione level, serum superoxide dismutase activity and serum ascorbic acid level. The maximum increase in blood glutathione level was obtained from part 2 extract (group 2). While part 1 and part 2 extracts (groups 1, 2) separately revealed a significant increase in SOD activity and ascorbic acid concentration more than that of the total herb extract (group 3), table 1.

Table 1: Mean blood glutathione content, serum ascorbic acid and serum superoxide dismutase among group of rats treated with 70 % alcoholic extracts of *T. alatus*.

Tested parameter	**Control Group**	**Group (1)**	**Group (2)**	**Group (3)**
Blood glutathione (mg/ gm Hb)	3.74 ± 0.064	4.93 ± 0.358*	6.3 ± 0.147*	5.11 ± 0.207*
Ascorbic acid (µg/ml)	3.56 ± 0.349	10.98 ± 0.56*	9.53 ± 0.328*	5.7 ± 1.43*
Superoxide dismutase (µg/ml)	17.6 ± 0.8	36.8 ± 1.46 *	24.5 ± 1.43 *	36.5 ± 1.6*

Group (1): Treated with 70 % alcoholic extract of part 1.
Group (2): Treated with 70 % alcoholic extract of part 2.
Group (3): Treated with 70 % alcoholic extract of total herb.
Results are expressed in mean ± SE
Number of animals in each group equals 6.
P (comparison with control) < 0.05 is significant.
* Significantly different from control value.

Table 2: Mean blood glutathione content, serum ascorbic acid and serum superoxide dismutase among groups of rats treated with different fractions of 70 % alcoholic extract of part 1of *T. alatus*.

Tested parameter	Control Group (4)	Group (5)	Group (6)	Group (7)	Group (8)
Blood glutathione (mg/ gm Hb)	3.74±0.064	5.46±0.272*	4.08±1.02	3.65±0.217	3.57±0.187
Ascorbic acid (µg/ml)	3.56±0.349	12.3±0.585*	11.95±1.17*	4.48±0.125	4.63±0.691
Superoxide Dismutase (µg/ml)	17.6±0.8	36.5±1.6*	36.8±1.46*	24.5±1.43*	24.5±1.43*

Group (5): Treated with chloroformic fraction of 70% alcoholic extract of part 1.
Group (6): Treated with ethylacetate fraction of 70% alcoholic extract of part 1.
Group (7): Treated with butanolic fraction of 70% alcoholic extract of part 1.
Group (8): Treated with ethanolic fraction of 70% alcoholic extract of part 1.
Results are expressed in mean ± SE. Number of animals in each group equals 6.
P (comparison with control) < 0.05 is significant. * Significantly different from control value.

Table 3: Mean blood glutathione content, serum ascorbic acid and serum superoxide dismutase among groups of rats treated with different fractions of 70% alcoholic extract of part 2 of *T. alatus*.

Tested parameter	Control Group	Group(9)	Group (10)	Group (11)	Group(12)
Blood glutathione (mg/ gm Hb)	3.74±0.064	4.0±0.095	6.0±0.294*	4.0±0.126	4.1±0.34
Ascorbic acid (µg/ml)	3.56±0.349	25.7±0.661*	14.8±1.56*	13.6±1.15*	13.5±1.35*
Superoxide Dismutase (µg/ml)	17.6±0.8	31.3±0.896*	27.5±0.668*	26.3±1.29*	25.9±0.973*

Group (9): Treated with chloroformic fraction of 70% alcoholic extract of part 2.
Group (10): Treated with ethylacetate fraction of 70% alcoholic extract of part 2.
Group (11): Treated with butanolic fraction of 70% alcoholic extract of part 2.
Group (12): Treated with ethanolic fraction of 70 % alcoholic extract of part 2.
Results are expressed in mean ± SE. Number of animals in each group equals 6.
P (comparison with control) < 0.05 is significant. *Significantly different from control value.

Regarding part 1 extract and its fractions, only the chloroformic fraction (group 5) revealed a significant increase in the blood glutathione level and showed no significant difference from the total extract (group 1). All fractions significantly increased the serum superoxide dismutase activity but the total extract (group 1) revealed the highest increase in SOD more than its fractions, this could be explained by the distribution of the constituent(s) responsible for the activity in the fractions, or could be attributed to synergistic effect of the compounds responsible for the activity in crude extract (Gordon 1995).

The chloroformic and ethylacetate fractions (group 5 and 6) exhibited a significant increase in serum ascorbic acid concentration and both fractions showed no significant difference from that of the total extract (group 1), table 2.

Regarding part 2 and its fractions , only the ethylacetate fraction (group 10) revealed a significant increase in the blood glutathione and showed no significant difference from that of total extract (group 2).

All fractions significantly increased the serum superoxide dismutase activity, but lower than that of the total extract (group 2).While all fractions significantly increase the serum ascorbic acid concentration more than that of total extract (group 2), table 3.

The antioxidant activity of *T.alatus* could be attributed to its flavonoidal content (Harborne *et al*., 2000). Flavonoids act as scavengers of various oxidizing species i.e. super oxide anion ($O_2^{-\bullet}$), hydroxyl radical or peroxy radicals, they also act as quenchers of singlet oxygen (Das *et al*. 1990). It was reported that carbonyl group at C-4 and the double bond between C-2 and C-3 are important features for high antioxidant activity in flavonoids, which is a common feature of the isolated flavonoids from the studied plant (Temraz *et al*., 2006).

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