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ANTICOAGULANT AND ANTIOXIDANT ACTIVITIES OF THYMUS VULGARIS IN DEXAMETHASONE TREATED FEMALE ADULT RAT

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ABSTRACT
This experiment aimed to investigate the anticoagulant and anti-oxidant activities of Thymus vulgaris (T.vulgaris) in hyper coagulation induced by long time Dexamethasone administration. Twenty four adult female rats divided into 4 groups; 1st Control group, 2nd and 3rd groups had subcutaneous injection with Dex 0.1mg/kg 3 times/week for 60 days, then 3rd and 4th groups received 75 mg/kg of T.vulgaris orally for two weeks. At the end of the experiment blood samples were collected via heart puncture from anesthetized rats. Results revealed that dexamethasone for 74 days caused significant ($p<0.05$) reduction in bleeding time, APTT and PT and increase in MDA and decrease in GSH and catalase enzyme. However treatment with T.vulgaris improve theses markers and elevated them to semi normal. Platelet count were elevated by dexamethasone and reduced by T.vulgaris. Liver function tests showed significant elevation in AST and reduction of total protein in Dexa groups. While decrease in T.vulgaris group. In conclusion, the anticoagulant activity of T.vulgaris against hypercoagultion induced by Dex mayight be through inhibition of intrinsic and extrinsic pathway as indicating PT and APTT tests for coagulation.

Keywords: bleeding test, catalase, prothrombin time, platelets, hyper coagulation

INTRODUCTION

Thymus vulgaris L. (thyme) is an aromatic, flowering plant typically grow as a sub-shrub from 5 to 30cm in height with fibrous ligneous root and small, greenish-grey leaves having narrow edged. It has numerous hard, branched stems (10-20cm tall) and flowers blooming from May to September having distinctive fragrance may white or purple in color. Due to its strong aroma that is because of thymol, it is grown widely as culinary herb (18). Local markets and traditional medicinal plant stores carry the plants that have historically been crucial in the treatment of human ailments all around the world (2), Due to the perception that natural additives, such as phytogenic and medicinal plants, as well as their derivatives, such as plant extracts or essential oils, are healthier, safer, and less regarded than synthetic additives, they have become more and more popular. (32) . Thyme possesses antibacterial, anti-inflammatory, and antioxidant properties. and anticoagulant activity and prevent platelet accumulation,according to many studies, it is clear that the principal pathways (intrinsic and extrinsic pathways) of the coagulation cascade are significantly affected by thymus species extracts. In this

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respect, previous works have reported the prolongation effect of polyphenol compound on the coagulation time (21, 3,5,), including rosmarinic acid and caffeic acid, the major phenolic compounds of tested Thyms aqueous extract (26), which mentioned in many works to possess a high effect of inhibition of coagulation time. Hence, these effects could be attributed to their high polyphenol content. Moreover, more studies are necessary to further investigate the active compounds of these plants and the mechanisms involved in action (7, 35). Dexamethasone (Dex) is a class of steroid drugs. (8) has potent immunosuppressant and anti-inflammatory properties and immuosuppresive. The immunosuppressive role primarily occur via inhibition of inflammatory cells and suppression of expression of inflammatory mediators (4).In spite of these properties, but therapeutic benefits of this drug limited as it presents several side effects such as insulin resistance and skeletal muscle atrophy and generation of the free radicals which may contribute to oxidative stress as long-term treatment (34). Although corticosteroid assisted the triclabendazole used in treatment of liver flukes (28), corticosteroids therapy associated with several forms of liver injury leads to increase the liver function enzymes (26). Glucocorticoids treatment may increase activation of coagulation producing a net change towards an increased risk of venous thromboembolism (17), increased circulating cortisol have been associated with severity of atherosclerosis. So the purpose of this study was to evaluate T.vulgaris role in haemostasis and antioxidant status balance against dexamethasone long period administration in adult female rats.

MATERIALS AND METHODS

Plant material

Thymus vulgaris plant were obtained from Suliamania Province-Iraq, and identified at Agriculture College/ University of Suliamania , Iraq. Plant leaves were drying and grinding. The 10% aqueous solution prepared by extraction of 30 gm dried powder of leaves in 300 ml DW using soxhlet for 5 hours (15).

Experimental Design

Twenty four adult (3-4 months age) female rat where divided equally into 4 groups and handled as followings: first group control: rats received normal diet and tap water , 2nd and 3rd groups received dexamethasone 0.1 mg/kg for 60 days three times a week , then the 3rd and 4th groups received T. vulgaris 75 mg/kg for two weeks according to (9). To ensure abnormal coagulators by dexamethasone, tail bleeding time were performed at the end of the 60 days by section of extremity of the tail tip 3 mm, the tail gently spotted with filter paper every 20 sec and the time in minutes cessation of bleeding (11). At the end of experiment blood samples were collected from anesthetized rats in sodium citrate anticoagulant tubes for hemostasis factors assay including; Activated partial thromboplastin time (APTT), Prothrombin time(PT) by Biomaghreb kit,(Tunsie). Platelets Large Cell Count (P-LCC), Platelets Large Cell Ratio(P-LCR) using an automatic analyzer device by Human device and kit(Germany). Other portion of blood transferred into gel tubes for serum isolation for estimation of Catalase, reduced glutathione (GSH), and malondialdehyde (MDA) Catalase, reduced glutathione, and

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malondialdehyde concentration by using ELISA kit My bio system (USA) and liver function tests including transaminases and total protein AST and ALT concentration and Total protein were measured by AGAPPE kit (USA).

Statistical Analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way, ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means.

RESULTS AND DISCUSSION

Hemostasis factors:

The statistical analysis of the results indicated that bleeding time(min),APTT (seconds), and PT(seconds) were significantly (P0≤.05) decrease in Dexa group, and significantly (P≤0.05) increased in rats
received 75mg/kg BW of T.vulgaris and, where there was no
significant (P0≤.05) differences in Dexa+T.vulgaris group when compare with-control(Table-1)

Table 1 The Effective role of T.vulgaris on Bleeding Time (minutes), Prothrombin Time (PT) (seconds), and Activated Partial Thromboplastine Time against Dexamethasone long treatment (APTT)(seconds). Means±SE, n=6

Treated Groups	Bleeding time (min.)	APTT (sec.)	PT (sec.)
Control	2.03±0.27b	12.33±0.71b	14.66±0.88b
Dexa. (0.1 mg/kg)	1.13±0.06c	7.83±0.70c	9.16±0.60c
Dexa. (0.1 mg/kg) +T. vulgaris(75mg/kg)	1.96±0.21b	9.33±0.71c	10.16±0.70c
T. vulgaris (75mg/kg)	14.90±0.29a	22.16±1.42a	28.50±1.17a
LSD	0.6781	2.7769	2.5619

The result in Table 2 shows that platelet count (PLT) and PLCC were significantly (P0≤.05) increased in dexamethasone group and significantly (P0.0≤5) decrease in T. vulgaris group and there was no significant differences in Dexa+T.vulgaris when compare with control group (Table 2)

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Table 2. The Effective role of T.vulgaris on Platelet count, Platelets Large Cell Ratio and Platelets Large Cell Count against the effects of Dexamethasone in female Rats. Means±SE, n=6

Treated Groups	PLT (10^9/L)	P-LCR (%)	P-LCC (10^9/L)
Control	553.16±70.37c	15.25±1.36	90.16±7.69bc
Dexa. (0.1 mg/kg)	675.83±83.78a	17.63±0.99	131.00±9.02a
Dexa. (0.1 mg/kg) +T. vulgaris(75mg/kg)	640.66±15.57b	16.60±1.49	95.00±4.85b
T. vulgaris (75mg/kg)	429.16±30.63d	15.04±0.53	71.66±3.97c
LSD	19.793	3.4193NS	19.793

Treated Groups

Regarding the side effects of dexamethasone when used for long term , the present results are in agree with results of Harris and his tem (12). Present results illustrated that long –term dexamethasone treatment significantly alter hemostatic parameters. Experimental studies have shown that glucocorticoid use can significantly increase the levels of clotting factors and fibrinogen (30) decrease fibrinolytic activity as indicated by increase fibrinogen (33) and increased platelet count (24). According to the present results result its clear that intrinsic and extrinsic pathways are affected by dexamethasone. T.vulgaris extract which is provide natural anticoagulant seems decreased bleeding time ,PT and APTT as it act as anticoagulant (10,13), Inflammation and coagulation are two main host-defense systems that interact with each other, Increasing evidence points to an extensive cross-talk between these two systems; indeed, inflammation activates coagulation and coagulation modulates the inflammatory activity in many ways (25).

Oxidant- Antioxidant balance

The results in Table 3 show that dexamethasone for 60 days caused significant($P \leq 0.05$) increase in MDA, on the contrary of T.vulgaris which caused significant reduction ($P0 \leq .05$) in this oxidant marker. On the other hand antioxidant markers, catalase and GSH were decreased significantly ($P \leq 0.05$) by dexamethasone and elevated by T.vulgaris

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Table 3. The Effective role of T.vulgaris on MDA, Catalase, and GSH against the effects of Dexamethasone in female rats. Means±SE, n=6

Treated Groups	MDA(nmol/ml)	Catalase(pg/ml)	GSH(mmol/ml)
Control	5.92±0.63b	206.58±25.75ab	10.61±2.38b
Dexa. (0.1 mg/kg)	15.04±1.01a	193.32±52.92b	7.74±1.01b
Dexa. (0.1 mg/kg) +T. vulgaris(75mg/kg)	13.65±1.43ab	223.64±22.42ab	8.39±0.85b
T. vulgaris (75mg/kg)	3.88±0.51c	298.52±15.58a	28.09±2.89a
LSD	2.8589	95.709	5.878

Hydroxyl radicals plus superoxide anions enhanced inflammatory cell infiltration in the portal area, accompanied by liver injury, may be attributed to DXM which induces cell membrane oxidative damage leading to fatty liver change (19). Regarding the oxidative stress, the existing findings agreed with Hasona and et al (13) showed that the major cause of liver injury induced by dexamethasone is oxidative stress.

Administration of thyme to dexamethasone intoxicated rabbits exhibited a significant decrease in malondialdehyde (MDA) as elevation in catalase and GSH. The recorded results may be related to the antioxidant properties of phenolic compounds found in the thyme (6, 16). This observation increase thoughts that the thyme have an effective protective mechanism in response to ROS and may be associated with decreased the oxidative stress and free radical mediated tissue injury. Decrease in (MDA) that thyme may protect the liver by preventing the increase of MDA because it scavenges the free radicals and this scavenging is one of the major antioxidant mechanisms to inhibit the chain reaction of lipid peroxidation agreement with Abu-Raghif and his team (1). On the contrary to dexamethasone that caused extreme formation of free radicals. DXM which induces cell membrane oxidative damage leading to fatty liver change (19). Elevated amounts of dexamethasone markedly reduced the actions of whole antioxidant capacity in addition to superoxide dismutase, resulting in oxidative stress by increasing the volumes of peroxidehydrogen plus malondialdehyde (21).

Liver Function Tests:

The specific liver function transaminases enzymes were affected by dexamethasone, ALT increased non significantly (P≤0.05) meanwhile AST increased significantly with significant decrease in total protein.T.vulgaris adiminstration keep these parameters in normal level in thymus group and restore to semi control level in dexa – Thymus group (Table4)

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Table 4. The effective role of T.vulgaris on ALT, AST, Total protein against the effects of Dexamethasone in female rats. Means±SE, n=6

Treated Groups	ALT (U/L)	AST (U/L)	Total protein (g/dl)
Control	28.88±1.29	13.32±0.78c	8.95±0.46a
Dexa. (0.1 mg/kg)	32.66±6.23	21.26±0.47a	6.46±0.28c
Dexa. (0.1 mg/kg) +T. vulgaris(75mg/kg)	30.84±1.89	18.08±0.64b	6.88±0.48bc
T. vulgaris (75mg/kg)	27.40±4.45	13.16±0.57c	8.08±0.64a
LSD	11.798NS	1.8584	1.4363

In the existing results,dexamethasone induced liver injury by increasing AST and ALT activities However,Thyme management effectively enhanced the elevated enzyme activities of the above-mentioned-parameters.These investigations are in agreement with other studies (14,31) whome declared that any harm in the liver can weaken its functions and cause numerous implications

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