

ANTICOAGULANT EFFECTS OF *EMINIUM SPICULATUM* AQUEOUS LEAF EXTRACT IN RABBITS

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ABSTRACT

The effect of different doses of *Eminium spiculatum* aqueous leaf extract on coagulation of blood in the rabbits were studied .Animals treated with extract at a dose of 200 , and 400 mg / kg body weight intraperitoneally daily for 7 days produced a significant ($p < 0.05$) increase in the clotting time , prothrombin time and activated partial thromboplastin time with normal platelet counts when compared with phosphate buffer saline (untreated) and heparin (treated) control rabbits. The effect of the extract on coagulation of the blood was shown to be dose – dependent . Result of this study suggest that extract shows considerable anticoagulant activity in rabbits and has potential to reduce cardiovascular morbidity and mortality.

INTRODUCTION

Laiyah (*Eminium spiculatum*) is a member of family *Araceae*.Its stout Cormous perennial herb with pedately dissected leaves , appearing together with the flowers. Spathe is very large upto 15 cm long , with an open , inside purple mottled tube ending in an inside blockish purple warty limb (1) .The plant is fairly common in the Mediterranean coastal region (2) . Many plants in this family are poisonous raw and if eaten raw , this toxin gives you sensation as if hundreds of tiny needles are sticking into the mouth tongue etc. Howerer , it is easily destroyed by thoroughly cooking (3) .The toxic principles in this plant are glycosides , calcium oxalate crystals packaged into bundles called raphides and proteolytic enzymes (4 , 5) . (3 , 6) recorded the plant contains a nicotinic like substances and triterpenoid .Some studies showed the plant can be used as insecticides (1) , antilipoperoxidative activity (7) , antimicrobial activity (8 , 9 , 10) and anticancer agent (11).Blood coagulation is a nother example of a process that must happen quickly when needed , but which has catastrophic effects if it occurs at an inappropriate time or location (12) . Blood coagulation, itself is a complex set of physical, cellular and biochemical events leading to thrombus formation (13) . Thrombus plays an important role in the pathogenesis and progression of atherosclerosis and cardiovascular disease (14 , 15) . The aim of this study is to examine the anticoagulant effects afforded by the aqueous leaf extract of *Eminium spiculatum* in rabbits.

MATERIAL AND METHODS

Animals:

The study was included (36) local breed rabbits , both sexes , 1 – 2 years age , body weight 1-1.5 kg.

Preparation of plant extract:

Eminium spiculatum fresh green leaves were used . The specimens were collected in Mosul city (Iraq) in late spring .The plant was properly identified. Fresh plant leaves were washed with distilled water . A 500 g of the plant leaves was cut into small pieces and blended using an electrical blender with 500 ml of 10 m *M* potassium phosphate buffer (PH 7.2). The mixture obtained was pressed through acheesecloth and the filtrate was centrifuged at 10000 xg for 1 hour. The supernatant fluid was separated and sterilized by filtration through nitrocellulose membrane (pore size 0.22 μ m) obtaining a clear solution , which was dried by lyophilization .Sterile extract were stored at – 20° C until used (16).

The study methods:

The animals were classified into six groups (each of 6 animals). Rabbits of the first , second , third , and fourth groups were treated intraperitoneally with aqueous leaf extract of *Eminium spiculatum* at a dose rate of 50 , 100 , 200 and 400mg / kg body weight respectively dissolved in 1 ml phosphate buffered saline daily for 7 days. Animals of the fifth group were treated with heparin 1 / kg b.w intravenously at day 7 of the study , and served as treated control , while animals of the sixth group were treated with 1 ml phosphate buffered saline (PBS) daily for 7 days and served as untreated control. Animals in the all groups were kept under observation daily and their laboratory changes were examined at day 7 of the study .

Samples:

1- Whole blood samples without anticoagulant :

Whole blood samples were collected from each animal of all groups in capillary tubes after venipuncture and clotting time was determined by the method of the capillary tube according to (12).

2- Blood plasma samples :

About 3 ml of whole blood samples were collected from each animals of all groups in dry clean tubes containing sodium citrate were used for measured the prothrombin time and activated partial thromoplastin time by using commercial standarad kits (Bio-Merieux , Baines , France) . Platelet count by using coulter counter and according to (12).

Statistical analysis :

Data were presented as means \pm standard error (SE) of mean .Differences between means were analysed by Students – t – test using Microsoft Excel computer software.Statistical probability of 5 % was used as a criterion for significance.

RESULTS

The results of observation revealed no apparent toxic signs or mortality in all groups. The results of is depicted in table 1. There is significantly increase ($P < 0.05$) of clotting time , prothrombin time and activated partial thromboplastine time in animals treated with extract at a dose of 200 and 400 mg / kg body weight intraperitoneally daily for 7 days when compared with PBS (untreated) and heparin (treated) control rabbits at day 7 of the study. The extract produced no significant changes in platelet count .

Table 1 : Anticoagulant effects in rabbits treated intraperitoneally with aqueous leaf extract of *Eminium spiculatum* at day 7 of the study.

Parameters	Groups of animals					
	Frist	second	Thrid	fourth	Fifth Ct	Sixth C
Prothrombin time (In second)	44.0±0.2	53.0±0.3	67.0±3.4*	81.0±3.1*	72.6±8.2	42.0±5.1
Activated partial thromboplastine time (In second)	12.4±2.1	18.7±2.4	25.3±2.3*	30.0±4.1*	27.2±2.2	11.8±1.7
Clotting time (In minute)	3.1±2.7	5.2±1.4	5.7±1.4*	6.2±2.1*	5.6±1.3	3.6±0.4
Platelet count $X 10^3 / mm^3$	232±12	258±13	267±23	274±12	256±31	243±41.1

* Significantly $p < 0.05 \pm SE$.

Ct : treated control.

C : untreated control.

DISCUSSION

The process of blood coagulation plays a central role in an organism's response to vascular injury on one hand and in cardiovascular diseases or thrombosis on the other hand (17). The treatment with any oral anticoagulants must be monitored to ensure that the dose is providing the required effects (18) Coagulation tests like prothrombin time ,activated partial thromboplastin time , thrombin time , fibrinogen time and platelet count. can better define the risk of bleeding. In present study , the aqueous leaf extract of *Eminium spiculatum* was shown to have anticoagulant effect in the rabbits. The results of laboratory test for coagulation revealed to signification increase in the clotting time , prothrombin time and activated partial thromboplastine time with normal platelet count , this effect were altributed to the toxic effect of the plant. The toxic principles in this plant are two , calcium oxalate crystals , proteolytic enzymes(4 ,5) , and triterpenoid (6) . This proteolytic enzymes causes inhibition of the blood clotting which may be result from its promoting the action of antithrombin III (19) . (20) recorded the effects of triterpenoid glycoside preparation on blood coagulation. Coumarin derivatives and several chemical analogs used to rodenticidal are known to cause bleeding problems in domestic animals (21) . These substances interfere

with vitamin K metabolism and therefore prevent synthesis of those coagulation factors dependent upon vitamin K (19).Other types of extracts of *Eminium spiculatum* showed its can be used as insecticides (1) , antilipoperoxidative activity (7) , antimicrobial activity (8 , 9 , 10) and anticancer agent (11)

CONCLUSION

The study showed that *Eminium spiculatum* affords a anticoagulant effect in rabbits. Futhermore studies to explain the active compounds contained in the extract. More over , there is a need to conduct detailed toxicity studies , using the plant on both laboratory and target animal species , to justify clinical investigation in other species of animals and in humans.

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التأثير المضاد للتخثر للمستخلص المائي للاوراق نبات اللاعيا في الارانب

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الخلاصة

درس تأثير عدة جرع من المستخلص المائي لنبات اللاعيا على تخثر دم الارانب . أدى معاملة الارانب بالجرع 200 و 400 ملغم لكل كيلو غرام من وزن الجسم عن طريق الخلب يوميا ولمدة سبعة ايام الى زيادة معنوية في زمن التخثر ، وزمن سابق الخثرين وزمن سابق الخثرين الجزئي الفعال مع بقاء اعداد الصفائح الدموية طبيعيا مقارنة بمجموعي السيطرة المعاملة بالملح الفسيولوجي والاخرى المعاملة بالهيبارين . لوحظ ان تأثير المستخلص يعتمد على الجرعة ، عليه اوضحت الدراسة ان للنبات تأثير مضاد لتخثر الدم في الارانب والذي ممكن استخدامه لتقليل الاصابة والهلاك من امراض الجهاز القلبي والوعائي.

REFERENCES

- 1-Al-Rawi, A. and Chakrararty, H.L. (1988). Medical plants of Iraq (2nd ed). Baghdad. Ministry of Agriculture and Irrigation.
- 2-Al-Qura'n , SA. (2010). Ethnobotanical and Ecological Studies of Wild Edible Plants in Jordan. Libyan Agriculture Research Center Journal Internation 1 (4): 231-243.
- 3- Huxly , A.(1992) . The New RHS Dictionary of Gardening MacMillan Press ISBN

- 4-Franceschi ,V. (2001) . Calcium oxalate in plants .Treds plant Sci. ,6(7):331.
- 5- Franceschi,V. and Paul, N.P. (2005).Annual review of plant biology., ,56:41-71.
- 6- Flayeh , K.A. Ahmad, T.Y.and Ali , Z.M. (1994) .Some metabolic effects of the toxic protin from *Eminium spiculatum* . Fitoterapia., 65(5):435-438.
- 7- Janakat , S. and AL- Thnaibat , O. (2008). Antilipoperoxidative effect of three edible plants extracts: *Viscum album* , *Arum dioscoridis* and *Emminiu Spiculatum*. Journal of Food Quality .31(1): 1- 12
- 8- AL- Younis, N and Abdullah , AF (2009). Isolation and antibacterial evaluation of plant extracts from some medicinal plants in Kurdistan region. J. Duhok Univ.12(1):250 – 255.
- 9- Darwish RM, and Aburjai T (2010). Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on *Escherichia coli*. BMC Comp. Alt. Med., 10: 1-9.
- 10- Obeida, M. (2011). Antimicrobial activity of some medicinal plants against multidrug resistant skin pathogens. Journal of Medicinal Plants Research .5(16): 3856-3860
- 11- Afifi , FU and Abu- Dahab, R (2011). Phytochemical screening and biological activities of *Eminium spiculatum* (Blume) Kuntzel (Family Araceae) Nat . Prod. Res. 31(2) :271 – 278.
- 12- Thrall, M.A.; Baker, D.C.; and Lassen ,E.D.(2004) .Veterinary Hematology and Clinical Chemistry .Lippincott William and Wilkins , London., , pp197.
- 13- Brummel KE, Paradis SG, Butenas S and Mann KG (2002). Thrombin functions during tissue factorinducedblood coagulation. *Blood*, **100**: 148-152.
- 14- Chandler WL and Velan T (2003). Estimating the rate of thrombin and fibrin generation in vivo during cardiopulmonary bypass. Hemostasis, thrombosis and vascular biology. *Blood*, **101**: 4355-4362.
- 15- Colman RW (2006). Are hemostasis and thrombosis two sides of the same coin *J. Exp. M.*, **203**: 493-495.

- 16- Benencia , F., Courreges, M.C. , Coulombie F.C. (2000) . In vivo and invitro immunomodulatory activities of *Trichilia glabra* aqueous leaf extract. J Ethnopharmacol. , 69,199-205.
- 17- Orfao SC, Jank G, Mottaghy K, Walcher S and Zerz E (2008). Qualitative properties and stabilizability of a model for blood thrombin formation. Journal of Mathematical Analysis and Applications, **346**: 218- 226.
- 18 -Zacchigna M, Luca GD, Cateni F and Maurich V (2004). Improvement of warfarin bio pharmaceuticals by conjugation with poly (ethylene glycol). European Journal of Pharmaceutical Sciences, **23**: 379-384.
- 19- Meyer, D.J.; Harvey , J.M. (1998) .Veterinary Laboratory Medicine : Interpretation and Diagnosis WB Saunders Company , London.
- 20- Kolkhir , V.K. , Sokolov,S.Y. (1982). Effects of triterpenoid glucoside preparations on blood coagulation .Pharmacol. Chemi. J.,16(5):335-340.
- 21- Radostits, O.M. , Gay , C.C. , Blood , D.C. , Hinchliff , K.W. .(2007) . Veterinary Medicine . Atextbook of the Diseases of Cattle , Sheep , Pigs , Goats and Horses . 10th ed. Philadelphia : WB Saunders Com.