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Research Article

In Vivo Anticoagulant Activity of *Meriandra dianthera* and its Sub-Acute Toxicological Effect on Hematological and Biochemical Parameters in Rabbits

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ABSTRACT

Extraction of potent bioactive compounds from medicinal plants permits the evaluation of their biological activity thereby aiding in the development of an effective drugs with less toxicity. The objective of this study was to evaluate the *in vivo* anticoagulant activity of *Meriandra dianthera* and its sub-acute toxicological effect on hematological and biochemical parameters in Rabbits. Adult age-matched white rabbits of both sexes were randomly divided into five groups (n=4). Rabbits of the first, second, and third groups were treated orally with an aqueous extract of *Meriandra dianthera* at a dose rate of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight respectively for 14 days. The control groups were treated with normal saline and heparin which served as negative and positive controls respectively. The animals in all groups were observed daily for signs of toxicity and mortality during the study period and their post extract administration effect on coagulation, hematological and biochemical parameters were examined. Rabbits receiving a crude extract of *Meriandra dianthera* at a dose of 400 mg/kg for the study period showed a significant increase in APTT, while the change in the PT value at all concentrations was found to be not significant. No adverse effects and also no significant change was observed in all the measured hematological and biochemical parameters after 14 days of the experimental period. It is therefore concluded that aqueous leaf extract of *Meriandra dianthera* has good *in vivo* anticoagulant activity and 14-days oral administration of the plant does not cause toxicity.

Keywords: Anticoagulant; *Meriandra dianthera*; Sub-acute toxicity; Hematological parameters; Biochemical analysis

INTRODUCTION

Blood clotting is a common and essential process to prevent bleeding from damaged blood vessels and thus maintain hemostasis by forming clumps or clots [1,2]. However, any abnormal clot formation in the circulatory system can potentially cause serious cardiovascular disorders. Anticoagulant drugs are required for the treatment of thrombotic disorders and to prevent their recurrence [3]. Drugs like heparins, vitamin K antagonists, and other derivatives are the commonly used anticoagulant drugs in the clinical setting. Despite having a good efficacy, these drugs have also been found to cause deleterious life-threatening side effects [4]. Therefore, the search for new potent alternative substances is necessary to replace the already existing anticoagulants and to reduce their associated side effects.

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially, those derived from plants [5]. Currently, despite progress in synthetic organic chemistry and pharmacology, the dependence on natural products, mainly on plants, remains essentially unchanged [6]. According to the WHO, 80% of the world's population primarily those of developing countries rely on plant-derived medicines for health [7].

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Medicinal plants have historically been the principal source of anticoagulant and antithrombotic molecules [8]. Despite the widespread use of medicinal plants their safety and effectiveness have not been entirely studied and more comprehensive screening is needed for evaluation and standardization of herbal formulations [9]. The widespread traditional use is not adequate to prove if herbal treatments are effective and safe. Therefore, like any other synthetic xenobiotic the use of medicinal plants should be based on supporting experimental evidence of the risks they present to their users. The two primary objectives of toxicological assessment of any herbal medicine are to identify the nature and significance of adverse effects and to determine limits of exposure level at which such effects occur [10-12]. Despite the popular traditional uses, the scientific validation of *Meriandra dianthera* has not been systematically examined. Therefore, this study was done to evaluate the *in vivo* anticoagulant activity and its sub-acute toxicological effect on the hematological and biochemical parameters in experimental rabbits. *Meriandra dianthera* (or its synonym name *Meriandera bengalensis*) is a fragrant shrub 50 cm to 2 m with dense branches. Most parts are covered with short grey hairs giving a white appearance. Its leaves are very aromatic abundant on hilltops and slopes where the soils are thin and rocky. It is also frequently found in abandoned farmlands 2,000-2,500 meters in the highlands. Traditionally its boiled leaves are used to treat colds, stomach aches, and hypertension [13].

MATERIALS AND METHODS

Plant collection and authentication

Meriandra dianthera leaves were collected from Adi-nfas, North of Asmara, Eritrea. A voucher specimen was identified by a botanist from the Eritrean Institute of Technology (EIT) and deposited at the Herbarium of Asmara College of Health Sciences, Department of biochemistry laboratory for future reference.

Sample preparation and extraction

The collected plant leaves were thoroughly washed with distilled water and were shade dried at room temperature for two weeks. The shade dried plants were then pulverized using mortar and pestle. 100 g fine pieces of *Meriandra dianthera* leaves were extracted for 5 h at 100°C with 1 L distilled water. Then the hot extract was filtered using grade number one filter paper then a rotary evaporator was used to concentrate the filtrate under reduced pressure. Finally, the semi-liquid product was further dried in a water bath at 60°C, and stored at -20°C until further analysis.

Study animals

Adult age-matched white rabbits of both sexes, weighing 2.4-3 kg were purchased from Paradizo local animal breading center. The animals were allowed to acclimatize for one week and fed with standard feed and water ad libitum throughout the study period. The animals were kept in separate cages and were housed under standard conditions at room temperature, humidity (40% to 50%) in an alternating 12-hour light/dark cycle. All experiments in this study were carried out according to the guidelines for care and use of experimental animals and the use of animals in this research and its experimental protocol was approved by the Asmara College of Health Sciences Research Ethical Committee [14].

Experimental design

The animals were classified randomly into five groups (n=4). Rabbits of the first, second, and third groups were treated orally with an aqueous extract of *Meriandra dianthera* at a dose rate of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight respectively, dissolved in normal saline (vehicle) daily for 14 days. Animals of the fourth group were treated with 1 ml/kg normal saline orally daily for 14 days, served as untreated control. Animals of the fifth group were treated with heparin 1 ml/kg (1 IU/kg) body weight intravenously on the 14th day of the study, served as treated control for the coagulation studies. The rabbit's weight was taken before and after the experiment. The animals in all groups were observed daily for signs of toxicity and mortality throughout the study period and their post extract administration effect on coagulation, hematological and biochemical parameters were examined 24 hours after the last treatment.

Blood collection procedures from rabbits

Blood sample was collected from rabbits of all the groups under aseptic technique after 24 hours of the last treatment. The ears of all rabbits were shaved properly using a blade to allow good visualization of vein and blood was collected from the marginal ear vein of the rabbits. The collected blood samples were used for the analysis of coagulation, hematological and biochemical parameters.

In vivo coagulation studies

For the coagulation study, the blood collected in light blue topped tube containing 3.2% tri-sodium citrate (BD vacutainer) was immediately centrifuged at 3500 rpm for 15 minutes to obtain platelet-poor plasma (HERMLE bench centrifuge). After centrifugation, the platelet-poor plasma was instantly separated in a clean plain tube for coagulation analysis. Coagulation analysis was done using an automated coagulometer (ACL elite coagulation analyzer, Instrumentation Laboratory Company). Prothrombin Time (PT) and Activated Partial Thromboplastin (APTT) were examined for all groups of rabbits and results were noted.

Hematological studies

The blood sample which was collected from the rabbits in lavender topped tube containing tri-potassium EDTA (BD vacutainer) was analyzed using an automated coulter counter (DXH 500). Measured hematological parameters include Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Hemoglobin (HGB), Hematocrit (Hct), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV) and platelet count.

Biochemical studies

For biochemical studies, the animals were fasted for 8 hours prior to sample collection and a blood sample was collected in a plain tube containing a clot activator (BD vacutainer). The sample was allowed to clot for 30 minutes at room temperature and then it was centrifuged at 3000 rpm for 15 minutes. The serum was separated into a plain tube and was analyzed using an automated chemistry analyzer (AU480 BECKMAN). The measured biochemical parameters included Alanine Amino-transferase (ALT), Aspartate Amino-transferase (AST), Alkaline Phosphatase (ALP), bilirubin, albumin, creatinine, blood urea nitrogen, cholesterol, glucose, triglyceride, HDL-C, LDL-C, calcium, and uric acid.

Data analysis

All the experiments were done in triplicate and results were presented as Means ± SEM. Statistical analysis was carried by using one-way Analysis of Variance (ANOVA) followed by Tukey's HSD test as the post hoc using SPSS software version 20. Values with $P<0.05$ were regarded as statistically significant.

RESULTS

Daily oral administration of *Meriandra dianthera* to the rabbits for 14 days did not produce mortality or any sign of toxicity like abnormal change in their fur, skin, eyes, mucous membrane, behavior pattern, tremors, salivation, diarrhea, convulsions, numbness, and coma. The change in the bodyweight of the entire treatment group was not statistically significant when compared to the control group.

Coagulation studies involving PT and APTT were performed after the 14 days of administration. Rabbits receiving a crude extract of *Meriandra dianthera* at a dose of 400 mg/kg for 14 days showed a significant ($P<0.05$) increase in APTT in comparison to control while the change in the PT value at all concentrations was found to be not significant (Figure 1).

The hematological parameters of the treated and control groups are presented in Table 1. No significant differences were recorded on all blood parameters measured after the administration of crude extract of *Meriandra dianthera* to the rabbits for 14 days.

The values for the biochemical parameters in treated and control rabbits are presented in Table 2. 14-days oral administration of aqueous crude extract of *Meriandra dianthera* in rabbits did not show any significant differences in the biochemical parameters between control and experimental groups.

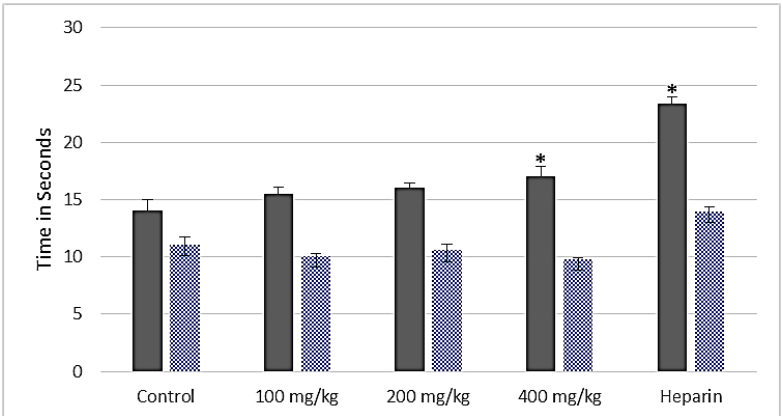

Figure 1: *In vivo* anticoagulant effect of the crude extract measured by the PT and APTT assays. (*=indicate significance at $P<0.05$), Note: (■) APTT; (▩) PT

Table 1: Effects of aqueous leaf extract of *Meriandra dianthera* on hematological parameters.

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg
WBC (103/µL)	8.46 ± 2.2	10.43 ± 1.1	11.23 ± 6.12	9.15 ± 1.76
LY (%)	47.2 ± 16.12	43.4 ± 8.4	37.5 ± 13.2	41.43 ± 5.7
MO (%)	6.5 ± 1.4	6.58 ± 1.6	7.4 ± 1.59	7.78 ± 2
NE (%)	43.8 ± 14.1	46.9 ± 6.1	51.7 ± 11.4	47.5 ± 8.23
EO (%)	1.91 ± 0.5	2.7 ± 0.8	2.8 ± 1.34	2.9 ± 0.44
BA (%)	0.09 ± 0.02	0.08 ± 0.03	0.06 ± 0.05	0.08 ± 0.013
LY# (103/µL)	3.73 ± 0.55	4.5 ± 1.01	3.5 ± 0.68	3.75 ± 0.78
MO# (103/µL)	0.58 ± 0.27	0.66 ± 0.12	0.86 ± 0.54	0.63 ± 0.18

NE# (103/µL)	3.94 ± 2.2	4.8 ± 0.77	6.2 ± 4.5	4.3 ± 1.3
EO# (103/µL)	0.17 ± 0.1	0.27 ± 0.06	0.33 ± 0.1	0.26 ± 0.06
BA# (103/µL)	0.01 ± 0.001	0.0083 ± 0.004	0.005 ± 0.0005	0.01 ± 0.0001
RBC (106/µL)	5.86 ± 0.09	5.53 ± 0.24	5.5 ± 0.25	6.45 ± 0.21
HGB (g/dl)	12.4 ± 0.5	12.4 ± 0.48	11.03 ± 1.88	12.91 ± 0.44
HCT (%)	38.8 ± 1.68	38.5 ± 1.6	34.5 ± 5.4	40.4 ± 1.74
MCV (fL)	62.3 ± 1.55	60.9 ± 0.92	64.1 ± 1.75	62.4 ± 1.69
MCH (Pg)	19.9 ± 1.02	19.4 ± 0.8	20.1 ± 0.82	19.5 ± 0.24
MCHC (g/dl)	31.93 ± 0.2	32.3 ± 0.4	31.9 ± 0.6	31.9 ± 0.63
RDW (%)	17.6 ± 1.25	17.8 ± 1.0	17.1 ± 0.8	16.95 ± 1.18
RDW-SD (fL)	25.1 ± 0.63	26 ± 2.8	26.7 ± 3.04	25.7 ± 0.6
PLT (103/µL)	211.5 ± 36.6	224.5 ± 24.5	259 ± 27.2	200 ± 59.3
MPV (fL)	5.9 ± 0.45	6.1 ± 0.43	6.1 ± 0.16	6.08 ± 0.2

Note: Values represent the mean ± S.E.M (n=4/group). WBC: White Blood Cells, LY: Lymphocytes, MO: Monocyte, NE: Neutrophils, EO: Eosinophil, BA: Basophil, RBC: Red Blood Cells, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RDW: Red Cell Distribution Width, RDW-SD: Red Cell Distribution Width-Standard Deviation, PLT: Platelet, MPV: Mean Platelet Volume

Table 2: Effects of aqueous leaf extract of *Meriandra dianthera* on biochemical parameters.

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg
BUN (mg/dl)	13.33 ± 2.08	12.33 ± 4.16	11.33 ± 0.5	13.33 ± 0.1.52
Ca (mg/dl)	11.5 ± 1.02	12.2 ± 0.8	12.5 ± 1.38	11.8 ± 1.04
AST (U/L)	43 ± 15.7	27.6 ± 3.2	52.6 ± 48.8	33.3 ± 3.5
TBIL (mg/dl)	0.16 ± 0.1	0.3 ± 0.001	0.3 ± 0.01	0.3 ± 0.01
ALB (g/dl)	3.63 ± 0.1	3.6 ± 0.15	3.8 ± 0.25	3.7 ± 0.3
CHO (mg/dl)	69.3 ± 28.9	86 ± 22.7	110.6 ± 42.8	76.6 ± 12.8
GLU (mg/dl)	125.6 ± 24.2	110 ± 9.5	114.6 ± 17.2	110.6 ± 38.2
IBIL (IU/ml)	0.26 ± 0.05	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.02
CREAT (U/L)	1.3 ± 0.2	1.16 ± 0.05	1.33 ± 0.25	1.23 ± 0.05
ALT (U/L)	72 ± 15.7	76.6 ± 19.5	65.3 ± 16.6	50.6 ± 19.5
ALP (U/L)	127.3 ± 21.7	106.3 ± 13.05	105.6 ± 32.08	90.6 ± 16.16
DBIL (mg/dl)	0.006 ± 0.005	0.0067 ± 0.005	0.02 ± 0.01	0.016 ± 0.005
TG (mg/dl)	56 ± 22.5	80.3 ± 42.6	63 ± 11	88 ± 52.8
HDL-C (mg/dl)	33.3 ± 10.1	30.3 ± 9.6	33 ± 12.1	26.6 ± 6.5
URIC (mg/dl)	0.26 ± 0.1	0.2 ± 0.1	0.23 ± 0.1	0.16 ± 0.11
LDL-C (mg/dl)	51.6 ± 21	39.6 ± 21.5	65 ± 28.2	32.3 ± 16.8

DISCUSSION

Extraction of bioactive compounds from medicinal plants allows the evaluation of their biological activity thereby aiding in the development of an effective drug with less toxicity [15]. In this study, the leave extract of *Meriandra dianthera* was examined for its *in vivo* anticoagulant activity and its sub-acute toxicological effect on hematological and biochemical parameters in rabbits after 14 days of exposure.

In the *in vivo* anticoagulant study, rabbits receiving a crude extract of *Meriandra dianthera* at a dose of 100 mg/kg and 200 mg/kg bodyweight for 14 days showed insignificant changes in all parameters in comparison to the negative control. However, a dose of 400 mg/kg for 14 days showed a significant increase in APTT in comparison to the control. The increase in PT was insignificant in all groups. The significant increase specifically in APTT indicates that crude extract of *Meriandra dianthera* may have shown its anticoagulant activity *in vivo* by specifically inhibiting the intrinsic coagulation pathway as reflected in APTT.

Acute toxicological assessment of *Meriandra dianthera* was previously done by on rats and found the plant to have an LD 50 value of greater than 2000 mg/kg [16]. However, no study has been done, to the best of the author's knowledge, on the plant's *in vivo* anticoagulant activity and its toxicological effect on hematological and biochemical parameters after 14 days of exposure to rabbits.

Biochemical examinations were performed on the rabbits to evaluate any toxic effects in kidney, liver, and glucose metabolism. The liver and kidney are the main organs exposed to the toxic effects of exogenous substances [17]. Serum enzymes like AST, ALT, and ALP are enzymes used as good indicators of liver function and as biomarkers for predicting possible toxicity [18]. These transaminases leak into the circulation when there is damage to hepatocytes [19]. The liver also controls the glucose synthesis from hepatic glycogen stores and is the major site of cholesterol synthesis and degradation [20]. As no significant changes were observed in AST, ALT, ALP, glucose, and cholesterol levels in this study suggest that 14-day oral exposure of *Meriandra dianthera* aqueous extract had no effects on the hepatocytes function and lipid or carbohydrate metabolism of the rabbits.

The effect of the *Meriandra dianthera* aqueous extract on the kidney was studied by assessing the serum level of creatinine and uric acid, which are considered essential indicators for kidney dysfunction [21]. An Increase in creatinine levels is mainly detected if there is marked impairment of functional nephrons [22]. Thus, the results recorded in this study suggest that the *Meriandra dianthera* aqueous extract did not affect renal function. All the other biochemical parameters showed no significant changes between the treated and control groups indicating that the plant doesn't affect or interfere with any of the measured biochemical parameters.

The hematological studies on the rabbits were performed to evaluate any toxic effect caused by aqueous extract of *Meriandra dianthera* on hematological parameters. Studies have revealed that when certain plant materials are consumed, they can either cause deleterious or stimulatory effects on the bone marrow and also in blood parameters [23]. Therefore, analysis of the hematological parameters is crucial in assessing the toxic effects of plant materials, as well as in determining the physiological and pathological status of the body, as changes in these parameters may indicate toxicity related to plant materials and various disorders like anemia, leukemia, reactions to inflammation and infections [24]. In this study there was no significant difference in all the measured blood parameters between the treated groups and the control group, indicating that the 14-days administration of hot aqueous extract of *Meriandra dianthera* had no effects on the circulating cells nor interfered with their production.

CONCLUSION

The results presented in this study demonstrate that the aqueous leaf extract of *Meriandra dianthera* has significant *in vivo* anticoagulant activity and also showed no toxicological effect on hematological and biochemical parameters in the rabbits after 14 days of exposure, which could also reinforce its potential. Further studies should be conducted to evaluate the plant's sub-chronic and chronic toxicological effect involving histopathological studies to assess its safety.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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