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Toxicological and Biochemical Studies of *Myristica fragrans* Hydroalcoholic Extracts in Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author AK designed the study, performed the laboratory tests, wrote the protocol, and wrote the first draft of the manuscript. Author MH performed the statistical analysis. Authors AK, VD and Author HRMB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Myristica fragrans* is one of the plants used as a herbal medicine. This plant includes some components that can treat many diseases. This study was done to evaluate the *M. fragrans hydroalcoholic* effects on some hematological parameters, thyroid hormones, some liver enzymes, blood urea nitrogen (BUN) and serum creatinine.
Study Design: This study was done on ninety adult male Wistar rats weighing approximately 200 to 220 g.
Place and Duration of Study: Nutmeg was authenticated in the Agriculture Faculty of Shiraz University (Shiras is a city in Fars province in the south -West of Iran). This study has been done in department of clinical sciences of faculty of Veterinary Medicine in Kazerun (Farsprovince, Iran) branch of Islamic Azad University, between March and September 2013.
Methodology: The *Myristica fragrans hydroalcoholic* extracts were administrated orally at 4 different doses (100, 200, 400 and 500 mg/kg BW /day) and in two terms (14 and 28 days) in

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experimental groups (groups A_1, A_2,A_3, A_4, B_1, B_2, B_3 and B_4). The control group did not receive extract. The Hemoglobin (Hb) concentration, packed cell volume percentage (PCV%), erythrocyte (RBC) and total leukocyte count (WBC), iron, total iron binding capacity (TIBC), ferritin, some liver enzymes, thyroid hormones, BUN and serum creatinine were assayed and compared to control group.

Results: The hydroalcoholic extract of *M. fragrans* have significantly increased effects on erythropoiesis and thrombopoiesis in the long-term and have significantly increased on T4 concentration in short and long periods ($P<0.05$) and decreased serum iron and TIBC. This extract did not have any significant effects on ferritin, and WBC, in short and long term ($P>0.05$). Liver enzymes activities, BUN and creatinine concentrations were not increased in any of experimental groups ($P>0.05$).

Conclusion: The hydroalcoholic extract of *M. fragrans* can be used for anemia, thrombocytopenia and hypothyroidism treatment with safe dosage and term, without any renal and hepatic side effects.

Keywords: Erythropoiesis; kidney; liver; thrombopoiesis; thyroxine; triiodothyronine.

1. INTRODUCTION

The *Myristica fragrans* which belong to *Myristicacea* family is one of the traditional medicine plants used in Asia for the treatment of some diseases, for example, stomach cramps, diarrhea and rheumatism [1,2]. The tree grows to about 30 m high with an undivided trunk. The leaves are alternately, dark green, entire – margined, sharp edged, short – petiole, ovate – elliptical, leathery and up to 8 cm long. The bark is a smooth grayish brown and the young branches are green. Male and female flowers are borne on separate trees, although there are male trees with female flowers and fruits [2]. Nutmeg has strong antioxidant properties and this correlates well with the total phenolic content. Macelignan is a component found in *Myristica fragrans* that has been reported to have a spectrum of pharmacological activities, including carminative, simulative, emmenagogue, antibacterial, anti-inflammatory, anti-obesity, larvicidal, anticancer, antidiabetes and hepatoprotective [3,4].

This plant at toxic doses can have many harmful effects such as the neurotoxicity in human, neuroblastoma SK-N-SH cell [5], convulusions, delirium and blurred vision, palpitations, nausea, dehydration and general body pain [6], these effects are due to *myristicin* content (5-allyl -1- methoxy -2,3-methylenodioxyben-ene) [7].

The results of some researches done on *M. fragrans* extract have been listed below:

The water extract of the leaves of *M. fragrans* have mitodepressive and antimutagenic potential as desirable properties of a promising anticancer agent [8].

The RP- HPLC method was successfully used to study the tissue distribution of MRL and the result explains the positive effects of nutmeg on the brain, liver and gastrointestinal tract [9]. Macelignan has the potential of type I allergy treatment [10]. *Myristica fragran* can be used in complex diseases treatment such as cancer and Alzheimer,s disease [4]. The *Myristica fragrans* Houtt increases both libido and potency, which might be related to its nervous stimulating property [11]. The extracts (50% ethanolic) of nutmeg and clove enhanced the sexual behavior of male mice [12].

Herbal medicinal products are unlikely to pose a significant threat to human health. Herbal medicines are required to meet the same safety, quality and efficacy criteria as any other licensed medicine, so it is important to have their appropriate doses. Thyroid hormones are necessary for normal growth because they increase body metabolism. These hormones are necessary for body improvement, mental and nervous system functions and maintenance of body temperature and energy. Liver and kidney are two of the most important organs that contribute in detoxification of drugs and toxins and their health is very important. In previous studies it has been reported that *M. fragrans* have anti-inflammatory effects [13,14]. According to the subject mentioned above about the importance of thyroid, kidney, liver and *M. fragrans* properties in this study, this plant,s hydroalcoholic extracts at the 4 doses(low, medium, high and very high) at two terms (14 and 28 days) were used and the effects of this extract on some hematological parameters, liver

enzymes, BUN, serum creatinine and thyroid hormones were investigated. Also, in this research an attempt has been made to find the dose and term which have most positive and lowest harmful effects.

2. MATERIALS AND METHODS

2.1 Extract Preparation

Nutmeg (dried kernel of *M. fragrans*) was purchased from a local herbal shop in Shiraz city (Shiras is a city in Fars province in the south - West of IRAN in the autumn and authenticated in the Agriculture Faculty of Shiraz University) . Then nutmeg was cleaned and powdered using an electric blender, and the powder was extracted with 70% alcohol for 72 h using a macerated method [15]. The resultant mixture was filtered through a Whatman no. 1 filter paper. The solvent of the filtrate was evaporated at ambient temperature, and the extracted powder was kept at 4°C until used and was dissolved in water before administration.

2.2 Experimental Animals

Blood samples were taken from the heart of ninety adult male Wistar rats weighing approximately 200 to 220 g divided into 9 groups of ten. Each group of rats was housed in separated standard propylene cages under standard laboratory conditions (12 h light–dark cycle and temperature 24-28ºC). Rats were kept in the animal house at the Kazerun (Fars province, Iran) Branch of Islamic Azad University and were fed a routine diet. The animals were divided into two general groups including groups A and B, belonging to two terms of study, 14 and 28 days respectively. These groups include: control group and experimental groups(groups A_1, A_2, A_3, A_4 that received hydroalcoholic extracts of *Myristica fragrans* orally for 14 days at the dose of 100, 200, 400, 500 mg/kg BW/day respectively and groups B_1,B_2,B_3,B_4 that received hydroalcoholic extracts orally for 28 days at the dose of 100, 200, 400, 500 mg/kg BW/day respectively). The control groups did not receive any extract.

2.3 Blood Sampling

In group A after 14 and in group B after 28 days all animals were weighed and their blood collected from the heart after anesthesia with ether. For determination of hematological parameters (Red Blood Cell (RBC), Platelet, total leukocytes count (WBC), hemoglobon (Hb) concentration, Packed cell volume (PCV) percentage and mean platelet volume (MPV) value) blood samples were collected into vacutainers containing ethyl-enediamine tetra-acetic acid (EDTA) as an anticoagulant. To determine biochemical parameters (iron, ferritin, total iron binding capacity (TIBC), liver enzymes, blood urea nitrogen (BUN), creatinine, Triiodothyronine (T_3) and thyroxine (T_4)) blood samples were collected into vacutainers and serum was separated by centrifugation at 750 *g* for 15 min and stored at - 20ºC until use. The samples with haemolysis were thrown away.

2.4 Hematological Parameter Assay

Packed cell volume (PCV) percentage and Hb concentration were measured using microhaematocrit and cyanmethaemoglobin [16] respectively. Red blood cell, Platelet and the total white blood cells were counted using the conventional method of Dacie and Lewis (2001). Blood was diluted with normal salin, Rees Eccher (1:200) and Marcano solution (1:20) for RBC, PLT. and WBC count respectively. MPV was determined by using below standard method. MPV (fl) = (Tct/Trc) x 10, where (Tct = platelet distribution index (PDW) x Trc Thrombocrit (Tct) = olume % of platelets Trc = platelet count)

2.5 Iron, Ferritin and TIBC

Ferritin was assayed by ELISA microwell method with kits of Delaware company. Iron and TIBC were measured by ferrozine and TIBC direct methods respectively with kits of Fortress Diagnostic company.

2.6 Thyroid Hormones Assay

Serum T4 and T3 were assayed by Radioimmunoassay (RIA) kits in the Namazi Research Center, Shiraz, Iran. The areas of validation for T3 and T4 assays including limits of detection and precision in standard curve following sample dilution, and inter- and intra-assay coefficients of variation results were considered. Intra-and inter assays for T4and T3 were below 6.2%, 8.6%, 3.3% and 8.6% respectively.

2.7 Liver Enzymes Assays

Serum activities of AST and ALT using Reitman Frankel and ALP by Bessey lowry Brock

methods (colorimetric standard methods) were assayed by spectrophotometer (Schimatzo, Japan) with Pars-Azmoon kits.

2.8 BUN and Serum Creatinine Assay

Serum concentration of creatinine and urea were assayed by Jaffe and urease kinetic methods respectively, by spectrophotometer method using Pars-Azmoon kits.

2.9 Statistical Analysis

Data were expressed in SI units and analysed using one way ANOVA method using SPSS/PC software [17]. Duncan's multiple range tests were used to detect significant differences between the means. All values were expressed as mean and standard error (SE) and $P<0.05$ was seen as statistically significant.

3. RESULTS AND DISCUSSION

The results of this study are observed in Tables 1-9. The results of this study showed at the dose of 400 mg/kg BW/day after 28 days (group B_3) of consumption of this extract there was a significant increase between the mean concentration of Hb, erythrocyte count and PCV percentage as compared to control group. At the dose of 200 mg/kg BW/day after 28 days (group B_2) significant increase was observed between the mean concentration of Hb and RBC count compared with control group ($P<0.05$).

At low dose after 28 days (group B_1) there was significant increase between RBC count as compared to control group($P<0.05$) (Table 3). There were significant differences between RBC count at the dose of 500 mg/kg as compared to doses 100, 200 and 400 mg/kg after 28 days (Table 3); also there were significant differences between RBC counts at doses of 100, 200 and 400 mg/kg at the term of 14 days in compartion to the 28 day period ($P<0.05$) (Table 9). Hydroalcoholic extract of *M. fragrans* reduced serum iron and TIBC over 14 days, so Significant differences were observed between serum iron at the dose of 500 mg /kg BW/day (group A_4) (Table 3) and TIBC at the dose of 400 mg/kg during this term (group A_3) in compared to control group ($P<0.05$) (Table 3). The decrease in TIBC levels after 28 days is higher than that of 14 days (Table 9) and there is significant decrease between TIBC after 28 days at the dose of 200, 400 and 500 mg/kg (groups B_2, B_3 and B_4) (Table 3). There were significant differences between the mean concentrations of iron at the dose of 400 with 200 and 500 mg/kg after 14 days (between group A_3 with groups A_2 and A_4) (Table 5) and between the same doses of two terms of sampling (after 14 and 28 days of *M. fragrans* hydroalcoholic extract consumption) ($P<0.05$) (Table 9). The statistical analysis indicated that at the dose of 400 and 500 mg/kg there were significant differences between TIBC of animals that received these extracts for 14 and 28 days (groups A_3 and A_4 as compared to groups B_3 and B_4) ($P<0.05$) (Table 9). There are no significant differences in total leukocytes counts between control and experimental groups ($P>0.05$) (Table 3). The hydroalcoholic extracts of *M. fragranse* increased the platelet mean count and decreased MPV. There were significant differences between the platelet mean count and MPV in rats that received 500mg/kg of this extract for 28 days (group B_5) compared to control group (group B_1). There was significant decrease in MPV at the dose of 200 and 400 mg/kg after 28 days of these hydroalcoholic extracts administration (groups B_2 and B_3) and also at the dose of 100 mg/kg after 14 days (group A_1) ($P<0.05$) (Table 3). The serum creatinine and BUN concentrations after the consumption of hydroalcoholic extract of *M. fragrans* at the dose of 100, 200, 400 and 500 mg/kg in two terms of this study did not increase (Table 2,4). The results obtained from this study expressed the hydroalcoholic extracts of *M. fragrans* did not increase ALT, AST and ALP activities (Table 2,4). The *M. fragrans* hydroalcoholic extracts increased T_4 concentrations at both term of administration, so that significant increase at the dose of 100,400 and 500 mg/kg after 14 days (groups A_1, A_3 and A_4) were observed; also at the dose of 200 and 400 mg/kg after 28 days (groups B_2 and B_3).This extract had significant increase effects on T_3 concentration in different experimental groups expect group A_2 (200 mg/kg after 14 days) (Table 4). In this study it was determined that the hydroalcoholic extracts of *Myristica fragrans* can increase erythropoiesis long term (after 28 days). RBC count be affected more than Hb concentration and PCV percentage. The RBC count increased after administration of this extract at the dose of 100 mg/kg for 28 days (group B_1), but Hb and PCV increased only in medium and high dose after 28 days.

The best dose of this extract for most erythropoiesis is 400 mg/kg (group B_3). In this dose RBC, Hb and PCV showed the greatest increase. It is interesting to note that in very high dose (500 mg/kg) erythrocyte increase is less than at the dose of 400 mg/kg and there were no significant differences between RBC count of group B_4 when compared to control group. The *M. fragrans* hydroalcoholic extracts have stimulating effects on erythropoiesis and this effect is related to the dose and term. Administration of high dose of *Fumaria officinalis* extract in long term may have suppressor effects on the immune system and induces anemia too, so it should be used cautiously [18].

In this study it was determined that the *M. fragrans* hydroalcoholic extract decreases serum iron concentration This finding could be perhaps related to the effects of erythropoiesis extract and the fact that iron consumption in erythropoiesis stimulation due to this extracta dministration is more than in nutmeg.

The statistical analysis showed the iron concentration changes have a direct relation to the dose and are indirectly related to the term of *M. fragrans* hydroalcoholic extracts administration. The concentration of Mg, Zn, Fe, Mn, Ca and Se were significantly decreased in serum of methylcholanthrene tumor models compared with the control and groups that received *Myristica fragrans* houtt [19].

The no increase in TIBC shows that serum iron reduction due to erythropoiesis increase is not as much as the TIBC increase result. In this research ferritin concentration did not change, indicating iron concentration decrease is lower than that affecting iron sources. This extract has no inflammatory effect, because its administration unchanged serum ferritin (one of the acute phase proteins) concentration and total leukocyte count. The increase in platelet count and decrease in MPV are some other results of this study. These changes at the dose of 500 mg/kg were significant, moreover MPV significantly decreased at the dose of 200 and 400 mg/kg after 28 days (groups B_2 and B_3) and at the dose of 100 mg/kg after 14 days (group A_1). These findings show this extract in the short term (after 14 days) just affects MPV in low dose, but in the long term (after 28 days) decreases at the medium, high and very high doses. In low dose this decreased was not observed.

The MPV decrease due to administration of this extract should be different from the MPV decrease that occurred due to some diseases for example: collagen diseases, schelolar and some neoplasmes such as breast cancer, Hodgkins disease, bronchogenic carcinoma. At doses in which only MPV decreased this diagnosis is easy, because in these diseases the MPV decrease is with platelet count increase, so in the highest dose after 28 days (group B_4) that platelet count increases with the MPV decrease, this determination is difficult.

According to the increasing effects of *M. fragrans* on erythropoiesis and thrombocytosis and no increasing effects on leukocytes, it seems this extract does not affect stem cells,but affects renal tubular epithelial cells and result in an increase in erythropoietin and thrombopoietin. The consumption of *M. fragrans* hydroalcoholic extracts had no harmful effect on kidney function, because it didn't significantly. Increase the serum concentrations of creatinine and BUN in any of the experimental groups. The intraperitoneal administration of Hypericum Perforatumhydroalcoholic extract at doses of 50, 100, 200 and 400 mg/kg had no significant effects on rats serum creatinine concentration [20]. *Hibiscus sabdariffa* (HS) consumption had no significant harmful effects on cholesterol, triglyceride, BUN, serum creatinine, Na and K levels in short term administration [21]. *Turnip* root alcoholic extract protects rats from renal degeneration [22].

Zataria multiflora hydroalcoholic extract affects the renal function in high dose as is evident by the significant changes in the creatinine test in animals that received this extract at the dose of 300 and 400 mg/kg, so it is better to use *Zataria multiflora* with caution, especially in renal disorders [23].

The administration of hydroalcoholic extracts of *Zataria multiflora* did not increase ALT, AST and ALP activities, so concluded consumption of this extract at the dose of 400 mg/kg and during a period of 28 days had no hepatotoxic effects [24]. Thyroxin concentration was increased in the experimental groups and there was significant increase in T_4 concentration in groups A_1, A_3, A_4, B_2 and B_3 as compared to control group. These findings show hydroalcoholic extract of *M. fragrans* can increase thyroid function after 14 and 28 days and the most effective dose is 400 mg/kg.

Table 1. The mean±SD of hematological parameters, iron, Ferritin and TIBC in control and experimental groups

Groups / Parameters	Mean±SD								
	WBC (103/µl)	Hb(g/dl)	RBC (10^6 /µl)	PCV (%)	Platelet (10^3 /µl)	MPV (Fl)	Iron (µg/dl)	Ferritin (ng/dl)	TIBC (µg/dl)
Control Group	6.70±4.10	11.85±1.76	6.64±0.84	34±4.7	382±149	7.0±0.21	295±132	0.60±0.28	389±40
Group A1a*	9.05±1.21	13.16±0.47	7.45±0.17	34±4.71	609±156	6.1±0.28	120±14	0.50±0.37	379±108
Group A2a*	6.33± 0.94	12.67±0.25	6.92±0.08	37±1.63	563±31	6.3±0.33	143±31	0.35±0.05	309±63
Group A3a*	5.62±3.11	12.12±0.16	7.19±0.30	36±0.42	561±95	6.5±0.49	131±31	0.38±0.13	327±28
Group A4a*	5.13±2.73	12.12±1.70	6.69±0.82	35.28±0.51	660±157	6.8±0.75	82±45	0.40±0.08	358±88
Group B1 b*	9.35±2.00	14.07±.14	7.77±0.12	35.05±4.70	630±65	7.0±0.57	233±35	0.90±0.14	295±4
Group B2 b*	9.85±1.83	14.62±0.24	7.94±0.21	38.72±0.42	697±40	6.6±0.21	211±13	1.45±0.07	236±11
Group B3 b*	6.12±1.22	14.72±0.31	7.98±0.24	40.02±0.34	689±70	6.7±0.31	212±23	0.75±077	224±30
Group B4 b*	6.52±1.20	14.04±0.53	7.45±0.25	40.20±1.11	918±133	6.52±0.48	240±19	0.65±0.49	221±35

**) The rats didn't receive received Myristica fragrans hydroalcoholic extract a*) Groups A1 ,A2 , A3 and A4:The rats received Myristica fragrans hydroalcoholic extract at the doses of 100 ,200 ,400 and 500 mg/dl BW respectively for the term of 14 days b*) Groups B1 ,B2 , B3 and B4:The rats received Myristica fragrans hydroalcoholic extract at the doses of 100 ,200 ,400 and 500 mg/dl BW respectively for the term of 28 days*

Table 2. The Mean±SD of biochemical parameters in control and experimental groups

Groups / Parameters	Mean ±SD						
	ALT (U/L)	AST (U/L)	ALP (U/L)	BUN (mg/dl)	Creatinine (mg/dl)	T_3 (ng/ml)	T_4 (µg/dl)
Control group	118±22	259±43	765±102	20.6±0.57	0.07±0.00	0.96±0.13	1.9±0.22
Group A_1	69±20	163±16	589±216	18.7±2.00	0.57±0.05	1.46±0.30	8.8±3.61
Group A_2	63±12	173±47	522±102	16.6±2.71	0.57±0.05	1.55±0.24	8.2±4.52
Group A_3	65±12	178±70	443±199	20.0±2.03	0.60±0.00	1.22±0.22	7.8±0.43
Group A_4	65±15	197±10	507±256	19.5±2.10	0.57±0.05	1.20±0.18	8.2±2.03
Group B_1	83±19	191±76	495±116	18.3±1.11	0.70±0.00	1.10±0.10	2.0±0.36
Group B_2	82±23	202±64	646±47	17.72±2.90	0.60±0.14	1.17±0.25	3.9±0.68
Group B_3	76±19	172±32	618±200	20.6±3.33	0.66±0.21	1.04±0.05	2.3±0.13
Group B_4	89±26	238±56	658±62	20.2±4.45	0.66±0.11	1.06±0.31	2.3±1.24

Table 3. Differentiation between hematological parameters, Iron, Ferritin and TIBC in control group in comparison with the two experimental groups

Groups / Parameters	WBC	HB	RBC	PCV	Platelet	MPV	Iron	Ferritin	TIBC
Group A_1	.56	.48	.40	.49	.20	.03	.15	.76	.88
Group A_2	.45	.63	.72	.66	.33	.05	.07	.42	.12
Group A_3	.73	.86	.52	.83	.10	.23	.16	.19	.04
Group A_4	.63	.86	.95	.87	.11	.74	.03	.50	.60
Group B_1	.40	.08	.03	.14	.82	.24	.47	.35	.05
Group B_2	.28	.00	.02	.07	.12	.02	.38	.13	.02
Group B_3	.38	.00	.01	.01	.22	.02	.39	.83	.00

Table 4. Differentiation between liver enzymes, BUN, serum creatinine, T_3 and T_4 in control group in comparison with experimental groups

Groups / Parameters	ALT	AST	ALP	BUN	Creatinine	T3	T4
Group A1	.06	.03	.29	.18	.01	.06	.03
Group A2	.01	.07	.03	.06	.01	.01	.07
Group A3	01	.15	.09	.60	.00	.15	.00
Group A4	.02	.06	.17	.40	.01	.13	.00
Group B1	.14	.27	.05	.03	a	.27	.72
Group B2	.15	.29	.18	.15	.28	.26	.00
Group B3	.09	.07	.34	.97	.76	.35	.01
Group B4	.25	.66	.24	.83	.48	.65	.62

a:There not any numerical and statistical difference between the mean concentration of creatinine in group B 1and control group

Table 5. Differentiation of hematological parameters, Iron, Ferritin and TIBC between different doses of *M. fragrans*e hydroalcoholic extract in the term of 14 days

(I) Group	(J) Group	Parameters								
		HB	PCV	RBC	WBC	Platelet	MPV	Iron	Ferritin	TIBC
		Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
Group A_1 (100Mg/kg)	Group A_2	.50	.47	.15	.03	.61	.63	.33	.31	.21
	Group A_3	.14	.20	.46	.14	.58	.30	.61	.38	.32
	Group A_4	.16	.19	.05	.11	.58	.09	.12	.49	.70
(200Mg/kg) Group A_2	Group A_1	.50	.47	.15	.03	.61	.63	.33	.31	.21
	Group A_3	.38	.54	.38	.25	.98	.54	.59	.82	.73
	Group A_4	.41	.48	.49	.39	.26	.17	.02	.73	.37
Group A_3 (400Mg/kg)	Group A_1	.14	.20	.46	.14	.58	. 30	.61	.38	.32
	Group A_2	.38	.54	.38	.25	.98	.54	.59	.82	.73
	Group A_4	.99	.89	.12	.79	.23	.38	.04	.88	.55
Group A_4 (500Mg/kg)	Group A_1	.16	.19	.05	.11	.58	.09	.12	.49	.70
	Group A_2	.41	.48	.49	.39	.26	.17	.02	.73	.37
	Group A_3	.99	.89	.12	.79	.23	.38	.04	.88	.55

Table 6. Differentiation of liver enzymes, BUN, serum creatinine and thyroid hormones between different doses of *M. fragranse* hydroalcoholic extract in the term of 14 days

(I) Group	(J) Group	Parameters						
		AST	ALT	ALP	BUN	Creatinine	T3	T4
		Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
Group A_1 (100Mg/kg)	Group A_2	.78	.62	.67	.22	1.00	.65	.79
	Group A_3	.67	.71	.36	.42	.38	.20	.68
	Group A_4	.33	.76	.60	.64	1.00	.16	.79
Group A_2 (200Mg/kg)	Group A_1	.78	.62	.67	.22	1.00	.65	.79
	Group A_3	.87	.89	.59	.05	.38	.07	.86
	Group A_4	.45	.84	.92	.10	1.00	.06	.99
Group A_3 (400Mg/kg)	Group A_1	.67	.71	.36	.42	.38	.20	.68
	Group A_2	.87	.89	.59	.05	.38	.07	.86
	Group A_4	.55	.94	.66	.74	.38	.88	.87
Group A_4 (500Mg/kg)	Group A_1	.33	.76	.60	.64	1.00	.16	.79
	Group A_2	.45	.84	.92	.10	1.00	.06	.99
	Group A_3	.55	.94	.66	.74	.38	.88	.87

Table 7. Differentiation of hematological parameters, Iron, Ferritin and TIBC between different dose of *M. fragrance* hydroalcoholic extract in the term of 28 days

(I) Group	(J) Group	Parameters								
		HB	PCV	RBC	WBC	Platelet	MPV	Iron	Ferritin	TIBC
		Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
Group B_1 (100Mg/kg)	Group B_2	.03	.07	.30	.67	.30	.20	.23	.30	.01
	Group B_3	.01	.03	.17	.01	.34	.27	.23	.76	.00
	Group B_4	.87	.36	.05	.03	.00	.11	.65	.62	.00
(200Mg/kg) Group B_2	Group B_1	.03	.07	.30	.67	.30	.20	.23	.30	.01
	Group B_3	.67	.78	.77	.00	.89	.78	.94	.21	.50
	Group B_4	.02	.01	.00	.01	.00	.78	.07	.16	.41
Group B_3 (400Mg/kg)	Group B_1	.01	.03	.17	.01	.34	.27	.23	.76	.00
	Group B_2	.67	.00	.77	.00	.89	.78	.94	.21	.50
	Group B_4	.00	.89	.00	.71	.00	.55	.07	.84	.87
Group B_4 (500Mg/kg)	Group B_1	.87	.36	.05	.03	.00	.11	.65	.62	.00
	Group B_2	.02	01	.00	.01	.00	.78	.07	.16	.41
	Group B_3	.00	.00	.00	.71	.00	.55	.07	.84	.87

Table 8. Differentiation of liver enzymes, BUN, serum creatinine and thyroid hormones between different dose of *M. fragranse* hydroalcoholic extract in the term of 28 days

(I) Group	(J) Group	Parameters						
		AST	ALT	ALP	BUN	Creatinine	T3	T4
		Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
Group B_1 (100Mg/kg)	Group B_2	.83	.96	.17	.82	.39	.66	.01
	Group B_3	.70	.70	.25	.38	.72	.71	.53
	Group B_4	.36	.74	.14	.46	.72	.80	.63
(200Mg/kg) Group B_2	Group B_1	.83	.96	.17	.82	.39	.66	.01
	Group B_3	.59	.74	.79	.23	.55	.37	.01
	Group B_4	.47	.70	.90	.30	.55	.44	.01
Group B_3 (400Mg/kg)	Group B_1	.70	.70	.25	.38	.72	.71	.53
	Group B_2	.59	.74	.79	.23	.55	.37	.01
	Group B_4	.21	.47	.70	.85	1.00	.88	.87
Group B_4 (500Mg/kg)	Group B_1	.36	.74	.14	.46	.72	.80	.63
	Group B_2	.47	.70	.90	.30	.55	.44	.01
	Group B_3	.21	.47	.70	.85	1.00	.88	.87

Table 9. Differentiation of the parameters studied at the same doses of *M. fragranse* hydroalcoholic extract in the terms of 14 and 28 days

Parameters	Groups		Sig. (2-tailed)	Parameters	Groups		Sig.(2-tailed)
T3	100 Mg/kg 200 Mg/kg	days 14-days 28 days 14	.12 .073	Hb	100 Mg/kg 200 Mg/kg	days 14-days 28 days 14	.01 0.000
	200 Mg/kg	days 14-days 28	.07		200 Mg/kg	days 14-days 28	.00
	400 Mg/kg	days 14-days 28	.11		400 Mg/kg	days 14-days 28	.00
	500 Mg/kg	days 14-days 28	.46		500 Mg/kg	days 14-days 28	.05
T4	100 Mg/kg	days 14-days 28	.03	RBC	100 Mg/kg	days 14-days 28	.03
	200 Mg/kg	days 14-days 28	.11		200 Mg/kg	days 14-days 28	.00
	400 Mg/kg	days 14-days 28	.00		400 Mg/kg	days 14-days 28	.00
	500 Mg/kg	days 14-days 28	.00		500 Mg/kg	days 14-days 28	.16
AST	100 Mg/kg 200 Mg/kg	days 14-days 28	.56	PCV	100 Mg/kg 200 Mg/kg	days 14-days 28	.40
	200 Mg/kg	days 14-days 28	.52		200 Mg/kg	days 14-days 28	.00
	400 Mg/kg	days 14-days 28	.90		400 Mg/kg	days 14-days 28	.00
	500 Mg/kg	days 14-days 28	.20		500 Mg/kg	days 14-days 28	.29
ALT	100 Mg/kg 200 Mg/kg	days 14-days 28	.44	WBC	100 Mg/kg 200 Mg/kg	days 14-days 28	.86
	200 Mg/kg	days 14-days 28	.22		200 Mg/kg	days 14-days 28	.00
	400 Mg/kg	days 14-days 28	.41		400 Mg/kg	days 14-days 28	.78
	500 Mg/kg	days 14-days 28	.18		500 Mg/kg	days 14-days 28	.40
ALP	100 Mg/kg 200 Mg/kg	days 14-days 28	.54		100 Mg/kg 200 Mg/kg 200 Mg/kg	days 14-days 28 days 14	.81 .002
	400 Mg/kg	days 14-days 28	.11	Platelet	400 Mg/kg	days 14-days 28	.00
ALP	500 Mg/kg	days 14-days 28	.30		500 Mg/kg	days 14-days 28	.04
	100 Mg/kg 200Mg/kg	days 14-days 28	.37		100 Mg/kg 200Mg/kg	days 14-days 28	.03

BUN	100 Mg/kg 200 Mg/kg	days 14-days 28	.77	MPV	100 Mg/kg 200 Mg/kg	days 14-days 28	.06
	200 Mg/kg	days 14-days 28	.63		200 Mg/kg	days 14-days 28	.21
	400 Mg/kg	days 14-days 28	.73		400 Mg/kg	days 14-days 28	.60
	500 Mg/kg	days 14-days 28	.76		500 Mg/kg	days 14-days 28	.45
Creatinine	100 Mg/kg 200 Mg/kg	days 14-days 28	.01	Iron	100 Mg/kg 200 Mg/kg	days 14-days 28	.00
	200 Mg/kg	days 14-days 28	.75		200 Mg/kg	days 14-days 28	.01
	400 Mg/kg	days 14-days 28	.55		400 Mg/kg	days 14-days 28	.00
	500 Mg/kg	days 14-days 28	.21		500 Mg/kg	days 14-days 28	.00
Ferritin	100 Mg/kg 200 Mg/kg	days 14-days 28	.23	TIBC	100 Mg/kg 200 Mg/kg	days 14-days 28	.25
	200 Mg/kg	days 14-days 28	.00		200 Mg/kg	days 14-days 28	.06
	400 Mg/kg	days 14-days 28	.62		400 Mg/kg	days 14-days 28	.00
	500 Mg/kg	days 14-days 28	.60		500 Mg/kg	days 14-days 28	.01

4. CONCLUSION

The *M. fragrans* hydroalcoholic extract have positive effects on erythropoiesis and thrombopoiesis, but do not have renal failure and hepatotoxic effects. This extract also stimulates thyroid function. It seems this extract has the most stimulating effects on Thyroid function and erythropoiesis at the dose of 400 mg/kg. At the dose of 500mg/dl, the most positive effect on platelet count was observed after 28 days.

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