

EFFECT OF *Salvia officinalis* and *Thyme Vulgaris* OIL ON HEMATOLOGICAL PARAMETERS OF INDUCED GASTRIC ULCER IN MATURE FEMALE RATS

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ABSTRACT

The present study has been carried out at the department of physiology, College of Veterinary Medicine of Basrah, to evaluate the role of oil extracted from *salvia officinalis* and *Thymus vulgaris* leaves in some structural and functional characteristics of mature female rats experimentally induced with gastric ulcer. Oils of thyme and sage were extracted from dry leaves available in Basrah market by normal hexane, oils components have been analyzed in a manner GC-MAS (Gas chromatography). Gastric ulcers were induced in starved female rats by giving indomethacin orally by mouth gavage in a dose 25mg/kg for one day. The animals have been divided into seven equal groups (8 females in each group). The first group was received normal saline and served as a negative control. Second group serve as positive group that received only single dose of indomethacin, whereas third group treated with thyme crude oil for two week, fourth group that treated with thyme dilution oil for two weeks, fifth group treated with sage crude oil for two week, sixth group treated with sage dilution oil for two weeks and seventh group treated with cimetidine for two weeks. The blood has been obtained for hematological assessment the present results: showed no changed in RBCs, PCV of female rats groups treated with thyme crude, thyme dilution, sage crude oil and cimetidine and just group treated with sage dilution caused a significant

decrease in RBCs and PCV among treated group. So, the results revealed that there is no change in WBC in groups treated with thyme crude, thyme dilution and sage dilution, whereas showed a decrease in gastric ulcer group, sage crude and cimetidine group. Also neutrophils increased significantly in all treated groups compared with control negative group, whereas no change recorded in thyme dilution and sage crude groups compared with control negative group.

INTRODUCTION

Gastric ulcer is a painful lesion that is caused by erosion of the mucosal layer of stomach or necrosis of the surface of the tissue due to sloughing of inflammatory necrotic tissue [1]. When the balance among aggressive factors such as acid, pepsin and defense mechanism such as (mucus, bicarbonate, blood flow, and mucosal turnover) is shifted in favor of aggressive factors, peptic ulcers are manifested [4]. Several mechanisms to explain the pathophysiology of gastric ulceration development associated with frequent NSAIDs use have been suggested, including inhibition of prostaglandin synthesis [2]. The non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin are capable of producing injury to gastrointestinal mucosa in experimental animals and humans and their use is associated with a significant risk of hemorrhage, erosions, and perforation of gastric ulcers [3]. The pathogenesis of Indomethacin-induced gastric ulcer such as leukocyte infiltration, free radical formation and disturbance in NO production in gastric tissues [4]. Herbal medicine that has been used for thousands of years to treat gastrointestinal distress such as *T. vulgaris* [5], *Salvia officinalis*. are a genus belonging to the family Lamiaceae and have been used as a medicinal plant against a variety of diseases, including gastric disorders and inflammatory process (6). The major constituents of *T. vulgaris* essential oil are thymol (23%–60%), α -terpinene (18%–50%), p-cymene (8%–44%), carvacrol (2%–8%), and linalool (3%–4%) [7]. Recent studies have revealed the gastroprotective activity of *S. officinalis* against ethanol-induced ulcers, via both free radical scavenging and inhibition of H⁺-K-ATPase pump [8]. It has been suggested that the protective mechanisms of *Salvia officinalis* against gastric stomach lesions are due to its antioxidant activity and the role it plays in gastric juice reduction [9]. Main components of *S. officinalis* are

phenol,2-methyl-5-(methylethyl) ,CarvacolAntioxidants,Isothymol,2-Methyl-Isopropyphenol.

Cimetidine tablets belong to group of medicines called H₂ receptor antagonist which act to decrease the natural acid secretion of stomach, is a histamineH₂ receptor antagonist that inhibits stomach acid [10].

MATERIALS AND METHODS

Preparation of oils extracts:-

The leaves of *Thymus vulgaris* (thyme), *Saliva officinali* (sage) were obtained in Basrah Province /Iraq. The dry plant leaves were cleaned and grounded, made a coarse powder by electrical grinder to get a uniform particle size and then used for extraction. seventy grams from all individual samples was defatted in a soxhlet apparatus, using 500 ml of hexane (boiling point of 40C°) for 3 hrs[11] .Essential oils diluted as oil/water emulsion with a concentration of (500 µl essential oil /ml) [12] crude oil as compared with cimetidine drug of choices .the emulsion were stabilized with 1% (Tween 80) [13],[14].

Animals preparation and management:

Fifty six healthy non pregnant female rats (*Lepuscuniculus*) weighed 180-200 grams body weight each were brought from the university of Thi-Qar\college of science. Female rats were kept for two week in the animal house of the College of Veterinary Medicine / University of Basrah, for acclimatization.

Induction of gastric ulcer:

The gastric ulcers were induced in forty eight starved rats by giving indomethacin (Safa co. Diala-Iraq) orally by using modified syringe and in dose 25mg\kg for one day . These forty eight rats were divided into six groups.

Experimental design

Group 1:served as control group (negative control) ,were given orally normal saline for 14 day.

Group 2: induced gastric ulcers by indomethacin 25 mg/kg in a single dose.

Group 3: indomethacin and treated with crude thyme oil 0.2mg/Bw for 14 days.

Group 4: indomethacin and treated with diluted thyme oil 0.1mg/Bw for 14 days.

Group 5: indomethacin and treated with crude sage oil 0.2mg/Bw for 14 days.

Group 6: indomethacin and treated with diluted sage oil 0.1mg/Bw for 14 days

Group 7: indomethacin and treated with cimetidine 20mg/kg for 14 days

Collection of Blood Samples

Blood samples (2ml) were collected from each animal at end of experiment by cardiac puncture. The (2ml) of blood was deposited into tube with anticoagulant which used for hematological analysis (RBC, WBC, Hb, MCV, MCH, MCHC, PCV and differential WBC

Statistical Analysis

The results were expressed as Means \pm S.D , all data were done with Statistical Package for Social Sciences (SPSS 11.0 for windows). The results were analyzed by using one way analysis of variance (ANOVA) between different treatment groups. Statistical significance was set at ($p \leq 0.05$).

RESULTS

From table(1) the revealed that RBCs and PCV improved in groups treated with Thyme crude, Thyme dilution, Sage crude and Cimetidine when compared with control(-ve) group, while the result in the same table showed there is a significant increase ($P \leq 0.05$) in gastric ulcer group(+ve) group when compared with control(-ve) group, and just group treated with sage dilution caused a significant decrease at ($P \leq 0.05$) level in RBCs and PCV among treated groups. From the same table it is clear MCV significantly increased in group treated with sage crude and sage dilution oil as compared with control(-ve) group, while the other treatment groups did not have any change when compared with normal group. On the other hand MCH and MCHC show significantly decreased in gastric ulcer group, thyme crude and cimetidine, when compared with control(-ve) group, whereas group treated

with sage crude showed a significantly increase in MCH and decreased in MCHC. Also groups treated with sage dilution and thyme dilution show significant decreased in MCH and and increased in MCHC.

Table 1 : Effect of Thyme, Sage oil and Cimetidine on RBC, PCV, HB, MCH, MCV and MCHC in female rats with gastric ulceration induced by indomethacine

Parameters Treatment	N O	RBC × 10 ⁶ /μL	Hb g/dl	PCV%	PLT g/L	MCV fl	MCH Pg	MCHC %
Control(-ve)Normal Saline(0.9% NaCl)	8	7.45± .21 A	14.05. ± 32A	42.64± 0.39A	750± 15.8 A	53.3± 3.17 A	53.303± 0.17A	32.35± 0.19 A
Control(+ve)Indomethacine(50mg/kg)	8	8.57± 0.35 B	14.22± 0.34A	46.10± 2.0B	732± 16.24A	54.85 ±0.76 A	17.21± 0.18 B	30.82± 0.35 B
Thyme crude(0.2mg/kg)	8	7.07± 0.79A	13.27± 0.29 B	40.53± 1.25A	796± 16.01A	54.68± 4.19A	17.60± 0.92B	31.83± 0.90C
Thyme dilution(0.1mg/kg)	8	7.45± 0.15 A	13.32± 0.15B	41.08± 0.10 A	767± 16.85 A	53.7± 30.89 A	17.65± 0.15 B	32.28± 0.31 A
Sage crude(0.2mg/kg)	8	7.28± 0.51 A	13.59± 1.06A	42.75± 3.72 A	884± 75.26 A	60.08± 0.8B	59.61± 0.87 D	31.65± 0.37 C
Sage dilution(0.1mg/kg)	8	6.46± 0.35 C	12.15± 1.04B	37.91± 3.19C	464± 794.2B	58.03± 8.69B	18.63± 0.64C	31.13± 0.50 A
Cimetidine(5mg/k)	8	7.30± 0.30 A	13.36± 0.31B	432± 1.01 A	852± 22.6A	54.99± 1.00A	16.60± 0.39 B	30.34± 0.31 D
LSD		0.51	0.68	3.11	167.5	4.73	1.42	0.48

N=number of animals., Capital letters denote differences between groups,P≤0.05 vs.

On the other hand, The obtained results in Table (2) revealed that those treated with thyme crude ,thyme dilution and sage dilution improved in WBC to reach to the normal level ,while gastric ulcer , cimetidine and sage crude groups showed a significant decrease in WBC as compared with control(-ve) group .From the same table we can show gastric ulcer group in addition to groups treated with thyme crude,sage dilution and cimetidine group showed a significant decrease in lymphocyte as compared with control (-ve) group , whereas group treated with thyme dilution and sage crude not has a significant change in lymphocyte as compared with control (-ve) group .However there is significant increase in

neutrophil in all treated groups as compared with control (-ve) group. On the other hand, monocyte increased in groups treated with indomethacine and thyme crude ,while showed a significant decrease in group treated with cimetidine and did not a significant changed in groups treated with thyme dilution, sage crude and sage dilution as compared with control(-ve)group, also groups treated with indomethacine, thyme crude and sage dilution show did not show any change in acidophil as compared with control(-ve)group, whereas decreased in groups treated with thyme dilution, sage crude and cimetidine. Further more, the basophil a significant increase in indomethacine ,thyme crude and sage dilution groups and did not have a significant changed in groups treated with thyme dilution, sage crude and cimetidine groups.

Table 2: Effect of Thyme, Sage oil and Cimetidine on WBC Counts and Percentage of Differential Count of WBC in Female Rats with Gastric Ulceration Induced by Indomethacin (Mean±SD)

Parameters Treatment	N O.	WBC× 10³/μL	Lymph %	Neut %	Mono %	Acido %	Baso %
Control(-ve)Normalsaline(0.9 % NaCl)	8	14.15 ±0.33A	84.73± 0.34 A	4.62± 0.46A	6.95± 0.11 A	3.16± 0.07 A	0.8± 0.07 A
Control (+ve)Indomethacine (50mg/kg)	8	8.86± 0.14B	63.87± 5.92 B	16.88± 0.24 B	11.52± 5.92 B	3.25± 0.28 A	4.41± 0.58B
Thyme crude(0.2mg/kg)	8	12.94± 2.74A	73.10± 5.25B	8.71± 5.90B	11.20± 8.71B	3.57± 1.11 A	3.41± 3.04B
Thyme dilution(0.1mg/kg)	8	11.94± 0.45A	84.47± 0.81 A	9.19± 0.14 B	4.85± 0.85 A	1.02± 0.07 B	0.48± 0.14A
Sage crude(0.2mg/kg)	8	8.39± 4.21B	83.05± 6.08 A	9.86± 4.81 B	3.38± 1.67 A	2.21± 0 74 B	1.48± 0.68A
Sage dilution(0.1mg/kg)	8	15.45± 3.75A	79.66± 5.61 B	11.16± 4.17 B	4.32± 1.07 A	2.63± 1.06 A	2.21± 0.54B
Cimetidine	8	3.95±	79.66±	17.35±	1.37±	1.62±	0.04±

(5mg/k		0.46C	0.45 B	0.13 B	0.12C	0.32 B	0.01A
LSD		2.51	4.70	4.08	4.25	0.93	1.41

N=number of animals., Capital letters denote differences between groups,P≤0.05 vs. control

DISCUSSION

the results of RBCs,PCV in gastric ulceration female rats by indomethacine showed a significantly increase in value this result meeting with result found by [15] , and did not agree with result found by [16] the main cause of different in results may be due to different in species of animals , dose of drugs and duration of treated. However Indomethacine prevent aggregation of platelets [17].

From the same table we can show the result of RBCs, PCV in group treated with thyme crude and thyme dilution oil improved to reach to normal as compared with (-ve) group these result documented by result found by [18] and [12]. Also there is increase in number of red blood cell ,PCV and in group treated with sage crude oil in compared with control (+ve)group these result are in agreement with [19] who showed the increase in red blood cell count occur due to positive effect of sage extract on kidney and increase in erythropoietin secretion[20].

MCV(mean corpuscular volume) measure of the average volume of a red blood corpuscle while MCH refers to the average quantity of hemoglobin that present in a single cell of RBCs.So there is a direct relationship between RBCs , PCV ,and heamoglobin concentration [21]. Indomethacin caused a significant decrease in the number of WBCs due to acute inflammation when they were compared with (-ve) control group This is a clear decreased because of gastric ulcer. This result covenant with(22) who reported prominont decrease in percentage of lymphocyte in acute inflammation.

While the increases in number of of neutrophil in indomethcin group compared with negative control group that consent with [23] who found the increase in

neutrophil is associated with a significant increase in leukotriene production which was observed 6 hours after dosage this may be due to acute inflammation.

On the other hand , the WBC of female ulcerated rats that treated with thyme crude, thyme dilution and sage dilution oils showed an improvement in immune system when it is compared with control(-ve) group through the increase in WBC number. The results compact with the results which confirmed the effectiveness of an anti-inflammatory thyme oil due to strong lipoxygenase inhibitory effects and because of thymol and carvacrol are the main components of the thyme oil humoral immune parameters indicated to elevation in lymphocytes percentage and neutrophils in blood by inhibited migration of them to the inflamed organ[17]. The Same results were reported by [18]. Whereas, white blood cell decreased significantly in group treated with sage crude oil as compared with control (-ve) group. These result agreed with[20] who conducted the reduction of WBC due to the affection stem cell that cause reduction in mitosis division , in addition affecting of progenitor cells and increasing mitosis division of them.

Oral administration of cimetidine did not causes a significant change in RBCs, PCV count. This result did not meet with result that found by [24] that explained cause of decreased RBCs count due to retarded haematopoiesis, destruction and shrinkage of RBCs it can be said that the decrease RBC counts may be due to haemolysis mediated via the chemical components of cimetidine, also Oral administration of cimetidine causes a significant decrease in WBCs count and the decrease observed may have resulted from suppression of leucocytosis (suppression of bone marrow to produce WBC)s by the drug and also from suppression of their production in the bone marrow these result meet with result found by[24].

Conclusion:The effect of thyme crude oil improved the gastric ulcer and blood parameters.

دراسة تأثير زيت الزعتر والميرمية على المعايير الدموية لاناث الجرذان الناضجة المصابة
تجريبيا بقرحه المعدة

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الخلاصة

اجريت الدراسة في فرع الفلسجة كلية الطب البيطري /جامعة البصرة، لتقييم الدور المحسن للمستخلص الزيتي لأوراق نبات المرمية والزعتر في بعض الخصائص التركيبية والوظيفية لإناث الجرذان البالغة المستحثة تجريبيا قرحة المعدة استخلصت زيوت المرمية والزعتر من الاوراق الجافة للنباتين المتوفرة في سوق البصرة بواسطة الهكسان. حللت المكونات الزيتية بكر وموتوكرافية الغاز و احدثت القرحة المعديه في اناث الجرذان المصومة باعطاء الاندوميثازين فمويا بواسطة المجرعة الفموية بجرعة ٢٥ ملغم/كغم ولمدة يوم واحد. قسمت الحيوانات الى سبعة مجاميع متساوية (كل مجموعته تحتوي على ثمانية اناث)، اعطيت المجموعة الاولى فقط محلول فسلجي واعتبرت مجموعة سيطرة سالبة، المجموعة الثانية اعطيت الاندوميثازين واعتبرت مجموعته سيطره موجب بينما المجموعه الثالثه اعطيت زيت الزعتر المركز ولمدة اسبوعين والمجموعة الرابعه اعطيت زيت الزعتر المخفف ولمدة اسبوعين اعطيت المجموعة الخامسة زيت المرمية المركز والمجموعة السادسة اعطيت زيت المرمية المخفف لمدة اسبوعين واخير المجموعة السابعة اعطيت السميتدين ولمده اسبوعين وتم سحب الدم للفحوصات الدموية وجدت النتيجة ان المعالجة بزيت الزعتر المركز والمخفف وزيت المرمية المركز ومجموعه السميتدين ادت الى تحسين كريات الدم الحمراء، وحجم الخلايا المضغوط ووصولها الى الحد الطبيعي بينما المجموعه التي اعطيت زيت المرمية المخفف ادت الى نقصانها، اشارت النتائج ان المجاميع المعامله بزيت الزعتر المخفف والمركز وزيت المرمية المخفف ادت الى تحسين بالكريات البيضاء ووصولها للحد الطبيعي بينما المجموعه المعالجه بالسميتدين والاندوميثازين وزيت المرمية المركز ادى الى نقصان بعدد الكريات البيضاء. كذلك اظهرت زياده معنوية في خلايا العدلة لكل المجاميع المعامله مقارنة مع المجموعه السيطره السالبة، بينما يسجل اي تغيير في الخلايا اللمفاوية لمجموعه الزعتر المخفف والمرمية المركز مقارنة مع مجموعته السيطره السالبة .

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