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Role of Haematological Indices, Interleukin-10, Tumour Necrosis Factor and Vascular Endothelial Growth Factor on Gastric Ulcer Healing in Obese Wistar Rats: Effect of Garlic Oil

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Abstract:- Haematological indices, cytokines and growth factors are among the factors that impact inflammatory processes. The impact of obesity on gastric ulcer (GU) healing is not fully known. Natural products like garlic oil (GO) which may be readily available and accessible choice, especially for the less privileged population are worth studying as regards its effect on gastric ulcer healing.

Male Wistar rats (80) weighing 120 g -140 g were randomly placed in 10 groups consisting eight each. Groups 1 to 5 were fed regular rat chow while groups 6 to 10 were fed high-fat diet (HFD) for eight weeks and weight were recorded weekly. By week 8, GU was induced with acetic acid in all rats except Groups 1 and 6 respectively. Rats in Group 2 and 7 were treated with normal saline (NS), Groups3, 4 and 5 were treated with 30mg/kg GO, 60mg/kg GO and 20mg/kg omeprazole respectively, while groups 8, 9 and 10 were likewise treated as above. Ulcer healing was assessed by measuring ulcer area by days 3 and 7 after ulcer induction. Ulcer area was significantly larger in obese group compared to negative control. Total white cell count was elevated in groups treated with garlic oil compared to normal control. GO increased level of interleukin-10, vascular epithelial growth factor and reduced tumor necrosis factor alpha levels. We conclude that garlic oil is beneficial for gastric ulcer healing in obesity state.

Keywords:- *Heamatological Indices; Interleukin-10; Obese; Tumor Necrosis Factor Alpha; Vascular Epithelial Growth Factor; Wistar Rats.*

I. INTRODUCTION

Gastric ulcer remains a prevalent gastrointestinal disorder with significant clinical and public health implications. Gastric ulcer is a chronic disease that results from an imbalance between endogenous protective factors of gastric mucosa and aggressive factors such as acid and pepsin secretions and reactive oxygen species [1-3]. Currently, proton pump inhibitors, H_2 receptor blocker, antibiotics for Helicobacter pylori clearance if present are used for treatment. Surgical interventions are also employed.

Natural products from plant sources have been used in treating many health conditions. Garlic products can be categorized under different categories like garlic powder, garlic oil macerate, aged garlic extract and garlic essential oil. Garlic has been studied for its medicinal and therapeutic effects in the treatment of various human diseases for a long time. Health benefits associated with the consumption of garlic are attributed to the various sulfur compounds present in it such as allicin, ajoene, vinyl-dithiin, and other volatile organosulfur compounds which are all metabolized from alliin. Several researches in the literature have shown evidence that garlic exhibits antioxidant, antiviral, anti-microbial, anti-fungal, antihypertensive, anti-anaemic, anti-hyperlipidemic, anticarcinogenic, antiaggregant and immunomodulatory properties [4-7].

Haematological parameters refer to the various characteristics and measurements of blood components that are assessed in a blood analysis. These parameters provide valuable information about the health and functioning of the blood and its cellular elements. Some of the haematological parameters include: packed cell volume, total white cell count, platelet count [8-10]. In a study on the ameliorative effect of nano chromium on some heamatological values and GLUT-2 in rabbits fed a high- fat diet, it was observed that there was a statistical rise ($p \leq 0.05$) in the number of erythrocytes, leukocytes, heamoglobin, and heamatocrit for the group of fat diet compared with the control [10]. People with obesity have a higher risk to undergo iron deficiency anaemia compare to lean people [11]. The characteristic of obesity as a systemic low-grade inflammation makes iron deficiency in the obese linked to inflammatory markers. Adipose-derived cytokines

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such as IL-6 and IL-1 act as potent inducers of hepcidin expression in adipose tissue. This elevation can decrease iron absorption and impair the effectiveness of iron fortification. In addition, obesity alters the lymphoid tissues, pro-inflammatory leucocyte distribution and population, and the amount of WBC, lymphocyte, neutrophil and monocyte. The WBC count will increase when the inflammation and leucocyte activation occurred [11].

Neutrophil to lymphocyte ratio (NLR) increases in local and systemic inflammatory conditions [12]. High NLR has been associated with poor prognosis in inflammatory processes. Increases in NLR indicate more inflammation is occurring [13]. Neutrophil to lymphocyte ratio in obese group of adolescents were significantly higher compared to those in healthy controls in another study [8].

Some of the used approaches to generate obese animals in different studies are diet (mainly high-fat diet), modification of genes and castration-induced obesity. The amount of calories from lipids in high-fat diet (HFD) ranges from 41 to 60% [14]. Obesity is considered when the body mass index (BMI) of the rat is greater than as follows: male (0.45 ± 0.02 - 0.68 ± 0.05) g/cm^2 and female (0.4504-0.50441) g/cm^2. The BMI is calculated using the following formula: body weight (g) /length^2 (cm^2) [15-17]. Obesity-induced systemic inflammation is triggered by immune cell recruitment, the interaction and activation of these immune cells, and the release of inflammatory molecules [18].

Cytokines are small secreted proteins released by cells have a specific effect on the interactions and communications between cells. Major anti-inflammatory cytokines include interleukin (IL)-1 receptor antagonist, IL-4, IL-6, IL-10, IL-11, IL-13 and TGF-β. Pro-inflammatory cytokines include; IL-1β, IL-6, IL-8, IL-9, IL-12, IL-15, IL-17, IL-18, IFN-γ, TNF-α and TNF-β. Increase in Interleukin 10 levels and decreased levels of levels of TNF-α, IL-6, IL-1β, and IFN-γ promoted healing of ethanol induced gastric ulcer in Wistar rats [19]

Vascular endothelial growth factor (VEGF) is a fundamental regulator of angiogenesis and is an endothelial cell-specific mitogen because its receptors are primarily restricted to endothelial cells [20,21]. VEGF binds to at least two specific receptors, VEGF-R1 and VEGF-R2, which are expressed mainly on endothelial cells, and initiates the phosphorylation of numerous cytosolic proteins involved in signal transduction that trigger endothelial cell proliferation, migration, and microvascular tube formation and angiogenesis [20,21].

Treatment with exogenous VEGF accelerated the healing of experimental gastric and duodenal ulcers in rats [22]. A local therapy with VEGF plus angiopoietin 1 or with serum response factor (SRF) plasmid dramatically accelerates gastric ulcer healing and improves the quality of mucosal restoration within ulcer scars [23].

In this study, the effect of haematological parameters, interleukin-10 and vascular endothelial growth factor on gastric ulcer area of obese male Wistar rats was assessed. This is to add to knowledge on gastric ulcer healing in state of obesity.

II. METHODOLOGY

Male Wistar rats weighing between 120 g and 140 g were obtained from the Animal house of the College of Health Sciences, Benue State University, Makurdi. They were housed in the same facility, under an environmental temperature of 23 ± 2^o C; humidity, 55±15% and 12 hour light/dark cycle. Water was given *ad libitum*. They were randomly assigned into 10 groups. Groups one to five were fed normal rat chow (Vital feed Limited, Kaduna, Nigeria) and groups six to ten were fed high fat diet with 60% energy from fat (tallow and soya oil) for 8 weeks. Animal handling and care was in line with the guidelines of the Institutional Research and Ethics Committee, which is also in line with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA). Ethical clearance for the use of animals was obtained from the Ethical Committee of the College of Health Sciences, Benue State University, Makurdi (CREC/THS/001).

The grouping of experimental animals and treatment were as follows:
Group 1 (Normal control): Normal rat chow + Normal saline.
Group 2 (negative control): Normal rat chow + normal saline
Group 3: (positive control): Normal rat chow + 20 mg/kg omeprazole
Group 4: Normal rat chow + 30mg/kg garlic oil (GO)
Group 5: Normal rat chow + 60 mg/kg garlic oil
Group 6: High fat diet (HFD) + normal saline
Group 7: High fat diet + normal saline
Group 8: High fat diet + 30 mg/kg garlic oil
Group 9: High fat diet + 60 mg/kg garlic oil
Group 10: High fat diet + 20 mg/kg omeprazole per oral

After 8 weeks of treatment gastric ulcer was induced in Groups 2 to 5 and Groups 7 to 10 for normal weight and obese rats respectively. No ulcer was induced in groups 1 and 6. Groups 1 to 5 were normal weight and Groups 7 to 10 were obese. Furthermore, feeding and treatment were administered orally.

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A. Preparation of Experimental Animals

After a 12 hour fast, each animal was anaesthetized with ketamine injection (80 mg/Kg) injected intraperitoneally [24].

B. Induction of Obesity Using High Fat Diet

The formulation of high fat diet was done according the method earlier described by Licholai *et al*., (2018) [25], with slight modification according to the method by Abi *et al*., (2020) [26].

Inclusion rate is as follows: From normal chow diet (growers feed) 60%, from tallow (25%) and soya oil (15%). The fat component comprised of 60% saturated fat and 40% unsaturated fat. The total caloric value of the diet was about 5340 kcal/kg of which energy contribution of fat was about 60%.

C. Weekly Weight Measurement:

The weight of the animals was recorded weekly using a digital electronic scale (Cocacina Co SF- 400C). The body mass index (BMI) was then calculated as follows:

$$BMI = \frac{Body\ weight\ (g)}{Nasoanal\ length\ (cm2)}$$

Normal BMI for male Wistar rats is 0.45 ± 0.02 - 0.68 ± 0.05 g/cm^2 and for female Wistar rats is 0.4504-0.50441 g/cm^2 [15-17].

D. Induction of Gastric Ulcer:

After 8 weeks of feeding and treatment, all rats were fasted for 12 hours before induction of ulcer. The animals were anaesthetized with ketamine (80 mg/kg) [24], and an upper midline laparotomy was carried out. the stomach was exposed, an area of the distal antrum was isolated with a clamp (using round forceps - ID 9 mm), and an acute gastric ulcer was induced by injection of 80% *v/v* acetic acid (0.04 mL) into the clamped portion. After 60s, the acetic acid was withdrawn, the serosa of the stomach was gently cleaned with normal saline, and the stomach was returned into the abdominal cavity. The abdomen was sutured back and the animals were placed in their cages after recovery with free access to food and water [13]. After ulcer induction, assessment of ulcer healing was done using four rats per group on days 3 and 7; the rats had the laparotomy sutures reopened and the stomach accessed for ulcers.

E. Measurement of Ulcer Area

By day 3 and 7, a total of 4 rats were randomly selected from each group for assessment of healing. The rats were sacrificed by cervical dislocation under anaesthesia**.** The stomachs were brought out, opened along the greater curvature, rinsed with normal saline and then spread out. Transparent paper was placed over ulcer area and the ulcer area was traced out. The area of ulceration was converted to units of square millimeters using 1mm by 1mm paper grid. Gross examination of the stomach was carried out with a lens to assess the degree of ulceration by looking out for lesions, haemorrhages, erosions, and thickening of the gastric epithelia. The percentage area of ulcer healed was calculated as [27]:

$$\%\ \text{of area healed on day 7} = \frac{\text{Area of ulcer on day 3 - area of ulcer on day 7}}{\text{Area of ulcer on day 3}} \times 100$$

➢ *Measurement of Haematological Parameters*

The full blood count was measured from the blood collected in EDTA bottles by cardiac puncture. An automated haematology analyzer (Beckman Coulter, DxH 900 haematology analyzer) was used. Packed cell volume, platelet count, neutrophil lymphocyte ratio were recorded.

➢ *Measurement of growth factors*

Measurement of the following was done using ELISA kit according to the manufacturer's instructions.

- *Tumor Necrosis Factor Alpha*

Rat TNF-α (Tumor Necrosis Factor Alpha) ELISA Kit, with Catalog No : E-EL-R0019 96T was used. This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in the kit is pre-coated with an antibody specific to Rat TNF-α. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Rat TNF-α and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Rat TNF-α, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Rat TNF-α. The concentration of Rat TNF-α in the samples is then calculated by comparing the OD of the samples to the standard curve.

- *Interleukin-10*

Rat IL-10 (Interleukin 10) ELISA Kit with Catalog No:E EL R0016 96T. This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit is pre-coated with an antibody specific to RatIL-10. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for RatIL-10and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain RatIL-10, biotinylated detection antibody and Avidin-HRP conjugate

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will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of RatIL-10. The concentration of RatIL-10 in the samples is calculated by comparing the OD of the samples to the standard curve.

- *Vascular Endothelial Factor*

Rat VEGF-A (Vascular Endothelial Cell Growth Factor A) ELISA Kit was used with Catalog No: E-EL-R2603. This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit is pre-coated with an antibody specific to Rat VEGF-A. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Rat VEGF-A and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Rat VEGF-A, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in colour. The enzyme-substrate reaction is terminated by the addition of stop solution and the colour turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Rat VEGF-A. The concentration of Rat VEGF-A in the samples is calculated by comparing the OD of the samples to the standard curve.

III. RESULTS

A. Gastric Ulcer Area Measurements of the Wistar Rats.

Table 1: Gastric ulcer area

Groups	Day 3 (mm^2)	Day 7 (mm^2)	% change in area
Group 1	0.00 ± 0.00	0.00 ± 0.00	0.00
Group 2	25.06 ± 1.13	14.5 ± 1.37	42.12
Group 3	13.56 ± 0.83	2.75 ± 0.25 #	79.72
Group 4	14.69 ± 0.64	6.13 ± 0.66 #	58.27
Group 5	15.44 ± 0.50	7.20 ± 0.98 #	53.37
Group 6	00.00 ± 0.00	0.00 ± 0.00	0.00
Group 7	31.5 ± 0.74 a	19.90 ± 1.65 #	36.82
Group 8	13.00 ± 0.40	5.9 ± 1.36 #	54.61
Group 9	13.94 ± 0.39	7.50 ± 1.07 #	46.20
Group 10	18.50 ± 2.65	11.0 ± 1.27 b #	40.50

Table 1 shows gastric ulcer area: Values are represented are mean ± SEM, N=4, a= significantly high at $p<0.05$ relative to negative control on day 3, b= significantly high compared to positive control $p< 0.05$. # = Significant at $p<0.05$ when compared with value by day 3.

Result showed that by day 3 after induction, ulcer area was largest in the obese rats that were treated with only normal saline (Group 7). By day 7, Group 7 had lower percentage change in ulcer area compared to negative control group 2.

The percentage ulcer healing was significantly higher in Group 3 (positive control) which were normal weight, ulcer induced, treated with 20 mg/kg omeprazole (79.72%). Treatment of both the obese and normal weight rats with garlic oil accelerated gastric ulcer healing and percentage healing for 30 mg/kg of garlic oil was higher than that for 60 mg/kg of garlic oil.

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B. Total White Cell Count

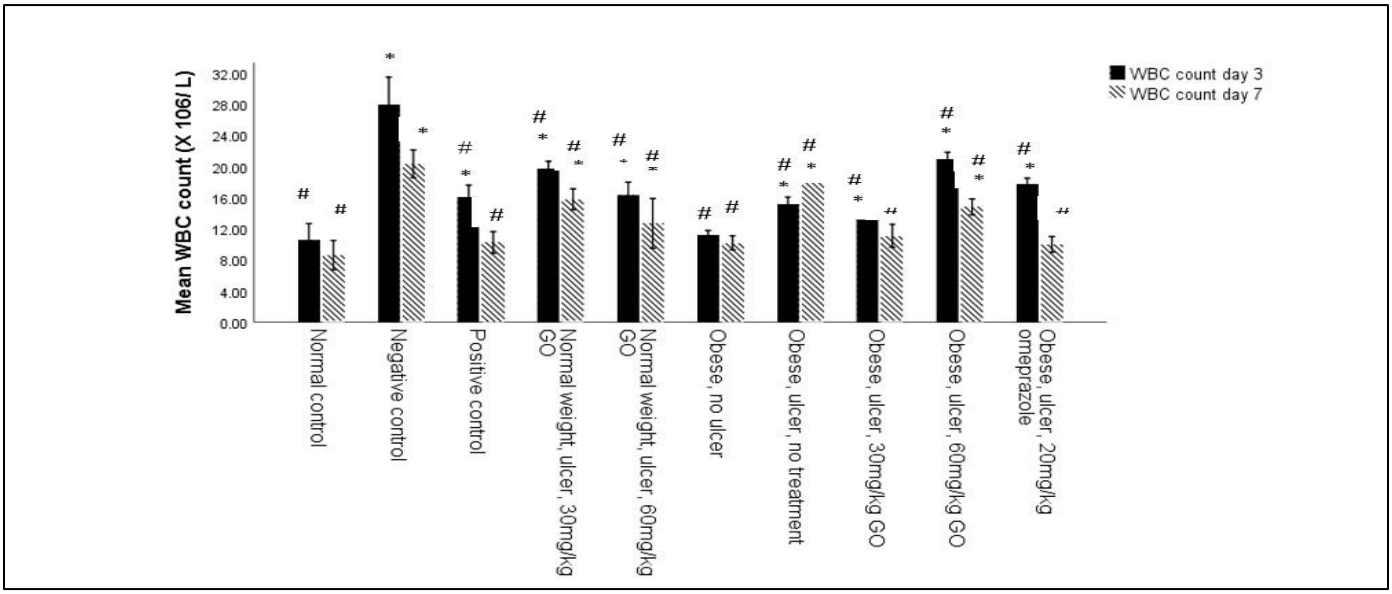

Fig 1 - Total white blood cell count

Figure 1 shows total white blood cell count- * Significantly higher relative to normal control ie group 1 (normal weight, no ulcer induced) on both days 3 and 7 at $p<0.05$, #= Significantly lower relative to negative control ie group 2 (normal weight, ulcer induced, no treatment given) on both days 3 and 7 at $p<0.05$.

Total white blood cell count (TWBC) was increased in the negative control ie Group 2 (normal weight, ulcer induced, no treatment). There was statistically significant difference between negative control ie Group 2 (normal weight, ulcer induced, no treatment) and Group 7 (obese, ulcer induced, no treatment). In Group 7, TWBC increased by day 7 while across all the other groups, TWBC decreased by day 7.

The groups that did not have ulcer induced (groups 1 and 6) had significantly lower WBC on both days 3 and 7 compared to all groups that had ulcer induced.

Administration of 30 mg/kg garlic oil in the normal weight groups showed higher TWBC compared to administration of 60 mg/kg of garlic oil. The reverse was observed in the obese groups.

C. Neutrophil Lymphocyte Ratio.

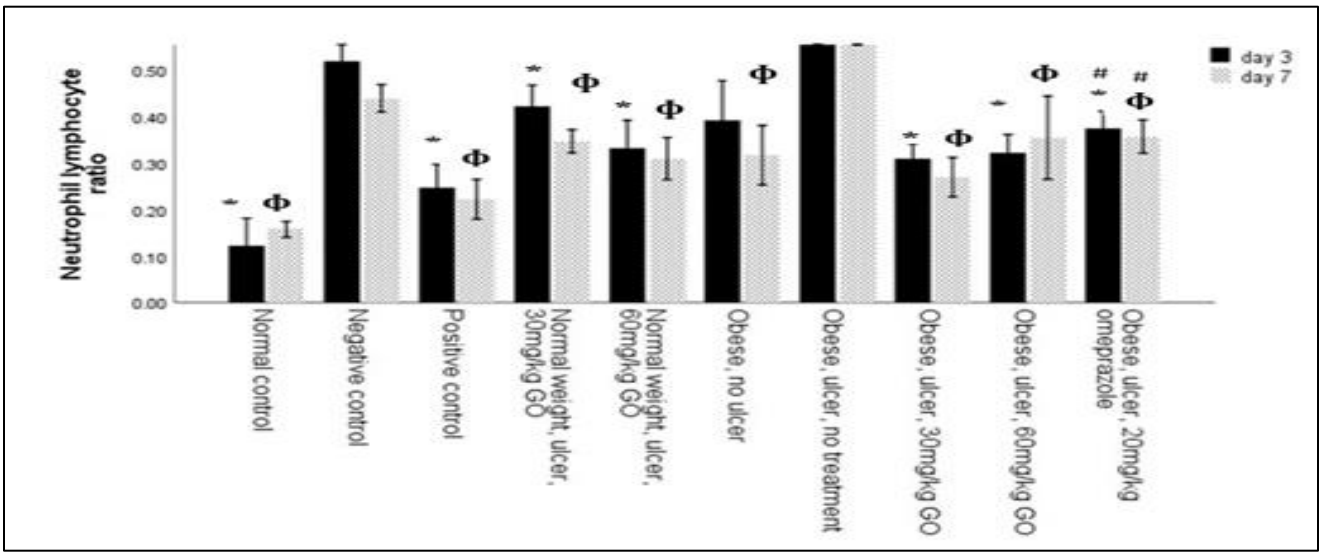

Fig 2- Neutrophil lymphocyte ratio

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Fig 2: Neutrophil lymphocyte ratio. * Significantly lower relative to negative control on day 3 at p <0.05, ϕ = Significantly lower relative to negative control on day 7 at p <0.05. #- Significantly higher to positive control on days 3 and 7.

Neutrophil to lymphocyte ratio was significantly higher in Group 7 (obese, ulcer induced, no treatment) compared to normal control, ie Group 1 (normal weight, no ulcer induced). In addition, the N/L ratio of Group 6 (obese, no ulcer induced) was significantly higher than normal control ie Group 1 (normal weight, no ulcer induced).

The positive control ie Group 3 (normal, ulcer induced, given omeprazole) had significantly lower NLR on both day 3 and day 7, compared to those treated with 30 mg Vs 60 mg garlic oil in the normal weight (groups 4 Vs 5) and obese (groups 8 Vs 9) Wistar rats.

There was no significant difference in NLR between the negative control ie Group 2 (normal, ulcer induced, no treatment given) and Group 7 (obese, ulcer induced, no treatment given). There was however, significant difference between the negative control and all other groups. By day 3, the NLR was higher compared to day 7 in all groups except group 7 (obese, with ulcer, no treatment).

D. Packed Cell Volume

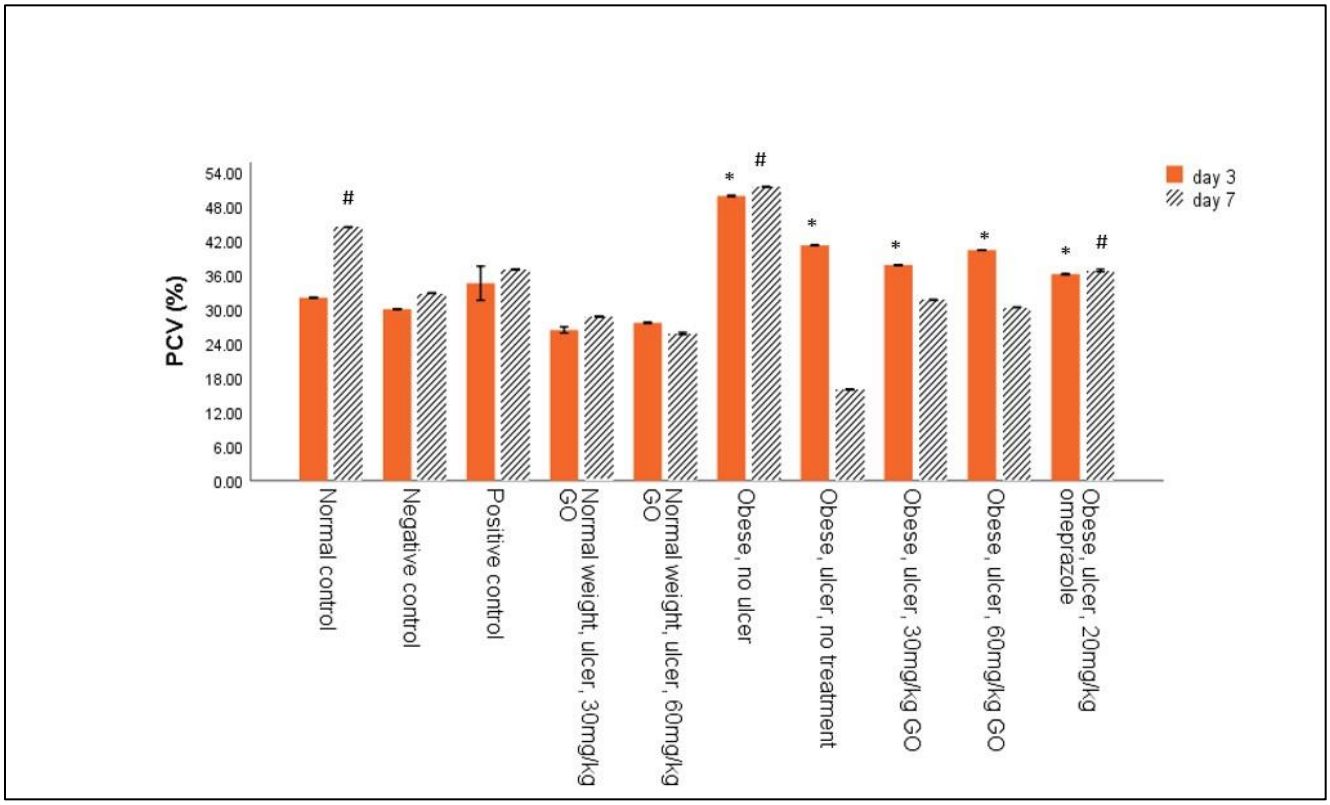

Fig 3: Packed cell volume

* significantly higher at p<0.05 relative to negative control on day 3, # = significantly higher at p<0.05 relative to negative control on day 7.

The packed cell volume by days 3 and 7 was significantly higher in Group 6 (obese, no ulcer induced). By day 7, the PCV of Group 7 was significantly lower than that of negative control, (normal, ulcer induced, no treatment given). The PCV of normal group that received 30 mg/kg and 60 mg/kg of garlic oil was lower than those of the obese groups on both days 3 and 7.

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E. Platelet

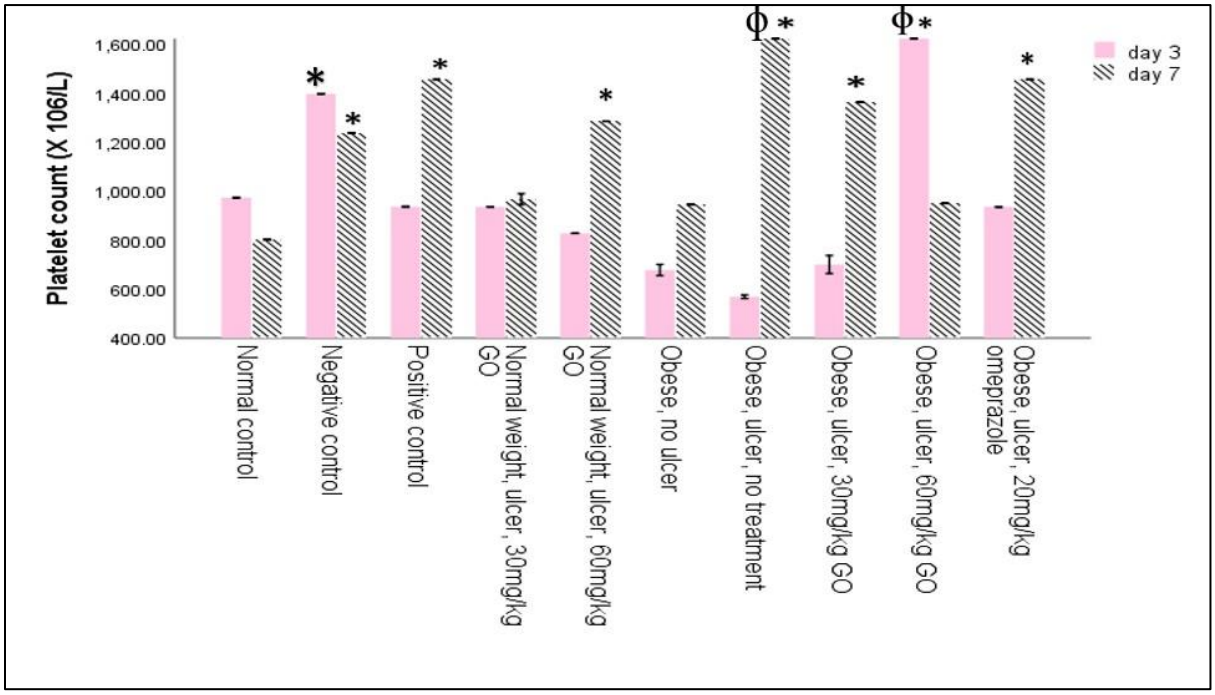

Fig 4 shows platelet count of the Wistar rats.
* Significant higher relative to normal control (Group 1) at P<0.05, ϕ= Significant higher relative to negative control (Group 2) at P<0.05.

The platelets count was significantly higher on day 7 in Group 7 (obese, ulcer induced, no treatment) compared to normal control. It was also high on day 7 for Groups 5, 7, 10 compared to negative control (Group 2). All these groups had ulcer induced.

F. Tumor Necrosis Factor

Table 2- Tumor necrosis factor

Groups	Tumor necrosis factor α (pg/ml)		Percentage change (%)
	Day 3	Day 7	
Group 1	148.21 ± 34.39	152.28 ± 26.29	2.75
Group 2	4374.19 ± 96.69	5834.70 ± 9.80	7.63
Group 3	391.82 ± 0.19	340.78 ± 44.01	-13.03
Group 4	534.83 ± 2.92[a]	523.13 ± 57.71[#]	-3.59
Group 5	651.07 ± 0.70[a]	622.55 ± 11.37[#]	-4.38
Group 6	306.46 ± 33.68[a]	293.58 ± 33.33[#]	-4.20
Group 7	7816.68 ± 44.74[b]	7960.06 ± 206.87*	1.83
Group 8	588.87 ± 3.33[a]	567.71 ± 87.26[#]	-2.19
Group 9	1709.11 ± 8.99[a]	1334.05 ± 16.81[#]	-21.94
Group 10	1793.10 ± 39.09[a c]	841.58 ± 2.92[# d]	-53.07

Table 2 shows tumor necrosis factor assay.
a-Significantly lower compared to negative control on day 3 at p < 0.05,
b- Significantly higher compared to negative control on day 3 at p< 0.05,
c- significantly higher compared to positive control on day 3,
- significantly lower than negative control on day 7 at p < 0.05,
* - significantly higher compared to negative control on day 7. d- significantly higher compared to positive control on day 7.

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Tumor necrosis factor (TNF α) was significantly lower by days 3 and 7 in both normal weight and obese groups compared to negative control group. There was significantly higher expression of TNF α in Group 10 (obese, ulcer induced, received 20 mg/kg omeprazole) compared to positive control (normal weight, ulcer induced, received 20 mg/kg omeprazole) by day 3 and 7 at $p < 0.05$. Group 7 (obese, ulcer induced, no treatment given) had the highest expression of TNF α and it was statistically significant. Percentage change in TNF α was significantly lower by day 7 in all groups except in negative control group (normal weight, ulcer induced, no treatment) and Group 7 (obese, ulcer induced, no treatment). On days 3 and 7 for both normal weight Vs obese groups, administration of 30 mg/kg garlic oil (group 4 Vs 8) showed reduced TNF α compared to administration of 60 mg/kg garlic oil (group 5 Vs 9).

G. Interleukin-10 Assay

Table 3 shows interleukin-10 assay

Groups	Interleukin 10 (pg/ml)		Percentage change (%)
	Day 3	Day 7	
Group 1	41.32 ± 0.12	37.56 ± 0.35	-9.10
Group 2	21.06 ± 0.19	23.52 ± 0.23	11.68
Group 3	241.65 ± 0.75	474.79 ± 0.40	96.47
Group 4	128.53 ± 0.34[a]	216.93 ± 0.05[#]	68.77
Group 5	104.98 ± 0.18[a]	136.66 ± 0.15[#]	30.17
Group 6	14.77 ± 0.06	14.52 ± 0.16	-1.69
Group 7	45.66 ± 0.10[a]	40.30 ± 0.20*	-11.74
Group 8	77.28 ± 0.75[a]	122.41 ± 0.34[#]	58.40
Group 9	100.37 ± 0.15[a]	122.40 ± 0.24[#]	21.95
Group 10	58.70 ± 0.08[a b]	67.71 ± 0.16 *[c]	15.34

Table 3: Interleukin -10 assay a-Significantly higher compared to negative control on day 3 at $p< 0.05$, b- significantly lower compared to positive control on day 3 at $p< 0.05$, # - Significantly higher than negative control on day 7 at $p< 0.05$ * - significantly lower than negative control on day 7 at $p < 0.05$, c- significantly lower compared to positive control on day 7 at $p < 0.05$.

Interleukin-10 was significantly higher on days 3 and 7 in both normal weight and obese groups compared to negative control (Group 2) at $p<0.05$. There was significantly lower expression of interleukin-10 in Group 10 (obese, ulcer induced, received 20 mg/kg omeprazole) compared to positive control (normal weight, ulcer induced, received 20 mg/kg omeprazole) on days 3 and 7 at $p < 0.05$. Percentage change in interleukin-10 was higher among normal weight groups compared to obese groups. On days 3 and 7 for normal weight groups, administration of 30 mg/kg garlic oil (Group 4) showed increased interleukin-10 compared to administration of 60 mg/kg garlic oil (Group 5). In the obese groups, the percentage change in interleukin-10 assay was higher on administration of 30 mg/kg garlic oil (Group 8) compared to administration of 60 mg/kg garlic oil (Group 9) on day 3.

H. Vascular Endothelial Growth Factor

Table 4 shows vascular endothelial growth factor assay

Groups	Vascular epithelial growth factor (pg/ml)		Percentage change (%)
	Day 3	Day 7	
Group 1	201.15 ± 38.39	204.64 ± 6.91	1.74
Group 2	121.70 ± 9.17	185.72 ± 1.95	52.60
Group 3	105.93 ± 11.61	180.68 ± 0.45	70.57
Group 4	184.24 ± 9.88[a b]	310.61 ± 5.76*[#]	68.59
Group 5	242.54 ± 33.92[a b]	385.69 ± 0.61*[#]	59.02
Group 6	361.87 ± 0.03	365.11 ± 5.49	0.89
Group 7	1690.36 ± 8.59[a b]	1197.44 ± 32.93*[#]	-29.16
Group 8	656.01 ± 5.87[a b]	530.53 ± 5.14 *[#]	-19.13
Group 9	554.58 ± 22.07[a b]	607.05 ± 14.18*[#]	9.46
Group 10	505.98 ± 0.30[b]	479.49 ± 0.31*[#]	-5.24

Table 4 shows vascular endothelial growth factor assay
a-Significantly higher compared to negative control on day 3 at $p< 0.05$,
b- significantly higher compared to positive control on day 3 at $p< 0.05$,

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- Significantly higher than negative control on day 7 at $p< 0.05$,
* - significantly higher than positive control on day 7 at $p < 0.05$.

Vascular epithelial growth factor (VEGF) was significantly higher by day 3 in the obese Groups 7, 8 and 9 but lower in normal weight Groups 4 and 5 compared to negative control. However, the percentage change in VEGF was higher for normal weight groups that received garlic oil (Groups 4 and 5) compared to obese groups (Groups 8 and 9) that received garlic oil. Vascular epithelial growth factor (VEGF) levels increased on day 7 across all treatment groups.

IV. DISCUSSION

In this study, gastric ulcer area, haematological parameters, tumor necrosis factor, interleukin-10 and vascular endothelial growth factor were measured among the study groups.

This study showed that healing was highest in the positive control group (normal weight, ulcer induced, treated with 20mg/kg omeprazole). The lowest percentage healing was seen in Group 7 (obese, ulcer induced, no treatment). There was decrease in ulcer areas across all groups on day 7 compared to day 3. Therefore, this study revealed that gastric ulcer healing was slower in obesity. This is in consonance with other studies where obesity impaired wound healing in burns, surgical wounds and chronic wounds [28-30]. In both the obese and normal weight groups, percentage healing for 30 mg/kg of garlic oil was higher than that for 60mg/kg of garlic oil. Garlic oil here, demonstrated ability to promote gastric ulcer healing in obese rats. Previous study has reported same in rat fed with normal chow [31,32]. Percentage ulcer healing was higher among the normal weight groups showing that obesity impairs gastric ulcer healing.

The total white blood cell count (TWBC) was significantly increased in the negative control (Group 2- normal weight, ulcer induced, no treatment) on day 3 which reduced by day 7. The subsequent reduction of TWBC by day 7 suggests abating of the ongoing inflammatory process and can encourage healing of the gastric ulcer. In Group 7, TWBC increased by day 7 while across all the other groups it decreased by day 7. This suggests a less optimal resolution of the inflammatory process in Group 7 (obese, ulcer induced, no treatment) compared to other groups. Other studies have also reported increase in TWBC during conditions of inflammation. For instance, the study on indomethacin-ulcerated rats treated with *Persea americana* seed and *Bryophyllum pinnatum* leaf ethyl acetate fraction demonstrated a significant elevation in the TWBC in rats with indomethacin-induced gastric ulcers [33]. In addition, patients who underwent distal gastrectomy for gastric cancer were found to have increased TWBC count by postoperative day one which reduced by postoperative week one [34].

The groups that did not have ulcer induced (groups 1 and 6) had significantly lower WBC on both days 3 and 7 compared to all groups that had ulcer induced which still buttresses that point that inflammation can contribute to elevated TWBC. However, there was no statistically significant difference between TWBC of normal control (normal weight, no ulcer induced) and group 6 (obese, no ulcer induced). Contrary to this, some studies have reported elevated TWBC among obese humans [35] and high fat diet fed mice [36].

Total white blood count was higher in all groups that were treated with garlic oil compared to normal control. The increase in total white cell count during acute inflammation is a dynamic response orchestrated by the immune system to combat pathogens, clear damaged tissues, and initiate the healing process. Administration of 30 mg/kg garlic oil in the normal weight groups showed higher TWBC compared to administration of 60 mg/kg of garlic oil. The reverse was observed in the obese groups and the reason for this observation need further study. The reason for the observation in the normal weight group may be attributed to the phenomenon of hormesis, where substances that are beneficial in small amounts can be harmful or less effective in large doses. Hormetic dose-dependent responses have been demonstrated in the use of plant products such as ginseng, curcumin, ginkgo biloba, and green tea and have potentially important public health and clinical implications [37]. Garlic oil, therefore potentiates acute inflammatory response at lower dose in normal weight Wistar rats compared to obese Wistar rats.

Neutrophil to lymphocyte ratio (NLR) was significantly higher in Group 7 (obese, ulcer induced, no treatment) and significantly lower in the normal control, Group 1 (normal weight, no ulcer induced). In addition, the NLR ratio of group 6 (obese, no ulcer induced) was significantly higher than normal control ie group 1 (normal weight, no ulcer induced). Thus, among the obese Wistar rats, NLR ratio was higher which may be attributed to systemic inflammation associated with obesity. This systemic inflammation is characterized by an adipose tissue driven acute-phase response, with interleukin (IL)-6, IL-1, IL-8, and tumor necrosis factor alpha (TNF)-α playing significant roles, resulting in subsequent elevations of acute-phase proteins such as c-reactive protein [38]. Neutrophil to lymphocyte ratio in obese group of adolescents were significantly higher compared to those in healthy controls in another study [8]. Similarly, neutrophil to lymphocyte ratio has also been reported to be increased in local and systemic inflammatory conditions [12]. In this study, the local inflammatory condition was induced gastric ulcer. High NLR ratio has been associated with poor prognosis in inflammatory processes. Increases in NLR indicate more

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inflammation is occurring [13]. This may explain poor ulcer healing in Group 7 (obese, ulcer induced, no treatment).

Treatment with 30 mg/kg and 60 mg/kg garlic oil in the normal weight and obese Wistar rats demonstrated lower NLR on both day 3 and day 7, compared to negative control, Group 2 (normal, ulcer induced, no treatment given). Hence, garlic oil at doses of 30 mg/kg and 60 mg/kg is beneficial in the healing of gastric ulcer.

There was no significant difference in NLR between the negative control, Group 2 (normal, ulcer induced, no treatment given) and Group 7 (obese, ulcer induced, no treatment given). There was however, significant difference between the negative control and all other groups. The NLR, is recognized as an indicator of systemic inflammation and immune status. While a high NLR is associated with poor prognosis in many diseases, a low NLR has been linked to favourable outcomes, particularly in terms of healing and recovery. Lower NLR has also been correlated with improved angiogenic responses, promoting adequate blood supply to injured tissues and facilitating healing [38].

The packed cell volume (PCV) on days 3 and 7 was significantly higher in group 6 (obese, no ulcer induced). On day 7, the PCV of group 7 (obese, ulcer induced, no treatment given) was significantly lower than that of negative control, (normal, ulcer induced, no treatment given). This shows that obesity is associated with higher haematocrit levels, but that in the presence of gastric ulcer, obese Wistar rats have lower PCV compared to normal weight Wistar rats. This will likely delay healing in the obese Wistar rat. Another observation of note in this study is that by day 7, Group 7 (obese, ulcer induced, no treatment given) had very low PCV compared to all other groups on the same day. It may be due to additive impact of presence of obesity and marked poorly abating acute inflammation in this group that did not receive treatment. During inflammation, erythrocytes can undergo both biochemical, as well as biophysical alterations. Biochemical changes can be seen as disruptions in the molecular arrangement of the bilayer, whereas biophysical changes are noted as changes to the general structural arrangement and erythrocyte morphology, translating to changes in erythrocyte mechanics. Inflammatory molecules interact with erythrocyte membranes which may lead to eryptosis which is commonly characterized by cell shrinkage and cell membrane scrambling [39].

The PCV of normal weight groups that received 30 mg/kg and 60 mg/kg of garlic oil was lower than those of the obese groups on both days 3 and 7. Unlike what was obtained in the above for group 7 which did not receive treatment, the finding on garlic oil treatment in the obese groups 8 and 9 may be explained as follows. In obesity, increased free radicals affect RBCs membrane protiens, increase fragility, decrease survival, cause anisocytosis and raise proportion of circulating premature erythrocytes.

To compensate for the reduction in red blood cell life, the body increases the production of new red blood cells, which then leads to an increase in RBC count in obesity [40,41].

The platelets count was significantly higher on day 7 in Group 7 (obese, ulcer induced, no treatment) compared to normal control. It was also high by day 7 for groups 3, 5, 8, 10. All these group had ulcer induced which is associated with inflammation. Elevated platelet has been observed to be high in obese state by other studies [9,42]. while in other studies, platelet count was not elevated by obesity [43,44]. The increase in platelets count in the groups with induced gastric ulcer can be attributed to reactive thrombocytosis which has been reported during inflammatory conditions and infection [45].

Platelets have long been known for their role in hemostasis, and currently attention has been drawn to their activities during inflammation. Inflammatory modes of function include interaction with leukocytes, secretion of proinflammatory mediators, migratory behavior, regulating macrophage functions and regulatory T cells, and secretion of pro-resolving mediators [46]. Platelets are known to interact with and enhance responses of regulatory T cells, resulting in increased IL-10 levels. Regulatory T cells are needed to support macrophage efferocytosis via secretion of IL-13 during resolution of inflammation [47]. Also, activated platelets themselves are known to modulate macrophages toward an anti-inflammatory phenotype with increased release of IL-10 and reduced secretion of TNF-α [48].

Tumor necrosis factor (TNF α) was significantly lower by days 3 and 7 in both normal weight and obese groups receiving either garlic oil or omeprazole compared to negative control group (normal weight, ulcer induced, no treatment given). This suggests that in the presence of gastric ulcer without any treatment (garlic oil or omeprazole) the expression of proinflammatory cytokine, TNF α is likely to be increased which can impair healing of gastric ulcer. Tumor Necrosis Factor α suppresses gastric microcirculation, cell proliferation, and angiogenesis at gastric ulcer margin, thus delaying ulcer healing [49,50]. Hence it may be said that reduction in the TNF-α level may enhance ulcer healing. Izhar, (2021), found that TNF-α levels increased in ethanol induced gastric ulcers in Sprague-Dawley rats [50].

Group 7 (obese, ulcer induced, no treatment given) had the highest expression of TNF α and it was statistically significant. Therefore, it is observed here that even with treatment with omeprazole, the obese Wistar rats had higher expression of TNF α. This aligns with the observation that, high fat diet caused an increase in the serum levels of TNF-α in mice as reported in another study [51].

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Interleukin-10 level was significantly higher by days 3 and 7 in both normal weight and obese groups that received garlic oil or omeprazole compared to negative control (Group 2). The lower IL- 10 level observed in the negative control group suggests that without treatment, in this instance with garlic oil or omeprazole, gastric ulceration results in reduction in IL- 10 level. Additionally, percentage change in interleukin-10 level was significantly higher among normal weight groups compared to obese groups, suggesting that HFD induced obesity may reduce IL- 10 level. Another study made a similar observation where HFD-induced obesity significantly depressed serum levels of IL-10 but increased several proinflammatory cytokines in both wild-type and IL-10 knockout mice [52].

By days 3 and 7 for normal weight groups, administration of 30 mg/kg garlic oil (Group 4) showed increased interleukin-10 compared to administration of 60 mg/kg garlic oil (Group 5). In the obese groups, the percentage change in interleukin-10 assay was higher on administration of 30 mg/kg garlic oil (Group 8) compared to administration of 60 mg/kg garlic oil (Group 9). This observation in this study where lower dose of garlic oil in some instances had more effect may help in clinical application in which dose titration can be applied to reduce side effect and reduce cost of procuring larger doses. Garlic oil was hence found in the present study to increase IL-10 level increasing the likelihood of healing of gastric ulcer.

Vascular epithelial growth factor (VEGF) was significantly higher on day 3 in the obese groups (Groups 7, 8 and 9) but lower in normal weight groups (Groups 4 and 5) compared to negative control. However, the percentage change in VEGF was higher for normal weight groups that received garlic oil (groups 4 and 5) compared to obese groups (groups 8 and 9) that received garlic oil. Therefore, in this study, there was higher VEGF levels among the obese groups of Wistar rats and is consistent with another study where serum VEGF concentrations were significantly positively correlated with BMI [53]. Vascular epithelial growth factor (VEGF) levels increased by day 7 across all treatment groups.

Vascular epithelial growth factor (VEGF) is involved in normal and pathological vessel formation. VEGF proteins induce an enhancement of sprouting as well as tube formation of vascular endothelial cells [22].

V. CONCLUSION

In conclusion garlic oil enhances gastric ulcer healing in obese Wistar rats. This experiment can be further applied on higher mammals to observe the effect of garlic oil on gastric ulcer healing obesity state.

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