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Gold Nanoparticles As Gastrointestinal Prevention For Escherichia Coli-Induced Colitis In Rats

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Abstract:
Background: *Colitis is worldwide distributed disease characterized by abnormal immune responses .Gold nanoparticles (GNPs) are recently used in medical field because their unique properties and have Low toxicity compared to other metallic .This study aimed to investigate the gastrointestinal protective ability of orally administrated Gold nanoparticles against E.coli induced ulcerative colitis in rats.* ***Methods:*** *E.coli isolated from cow's milk with mastitis and used to induce ulcer in rats. Waster albino rats was administration orally GNPs at two doses 5µg/Kg & 10µg/Kg as a prevention factor against E.coli induced ulcer compared with ulcer group and group administration omeprazole and evaluated the immunological effect of GNPs measured the level of SOD, MDA, TNF –alpha, TGF-β and PGE2 by ELISA methods from the blood rats.* ***Results:*** *The result shown that GNPs have protective effect against induced ulcer as dose dependent manner and have antioxidant properties through elevation of the SOD level and decrease the level of MDA compared with ulcer and omeprazole groups . Although the GNPs modulating the immune response by lowering the level of TNF –alpha and the level of transforming growth factor (TGF-β), and PGE2 significant compared with ulcer and omeprazole groups.* ***Conclusion:*** *Gold nanoparticles has the effective ability of gastroprotective against bacterial induced ulcer through the enhancement antioxidant enzyme and modulation the proinflammatory as TGFb and PGE lead to decreased tissue injury and inflammatory cytokines TNF-alpha.*
Key words: *Gold nanoparticles, Ulcer, prevention, Immune modulation, TNF –alpha, PGE2*

INTRODUCTION

Antibiotic resistance is set to become one of the greatest threats to human existence and new treatments or more effective ways of treating infections have to be developed. The prevalence of antibiotic resistance is quickly becoming one of the world's greatest health challenges with predictions of over 10 million deaths worldwide by 2050 [1]. Nanotechnology has extensive application as nanomedicine in the medical field. Some nanoparticles have possible applications in novel diagnostic instruments, imagery and methodologies, targeted medicinal products, pharmaceutical products, biomedical implants, and tissue engineering. Today treatments of high toxicity can be administered with improved safety using nanotechnology, such as chemotherapeutic cancer drugs [2]. More than 4.9 million people globally suffer from inflammatory bowel diseases (IBDs), which include Crohn's disease and ulcerative colitis [3].. The clinical presentation depends on disease location and may include diarrhea, abdominal pain, fever, clinical signs of bowel obstruction, and anal passage of blood, mucus, or both [4]. The exact etiology of IBD is still unclear, but studies indicate several possible links to genetics , immunology , nutrition , bacteria , viruses and other environmental factors . Animal model studies suggest that inflammation in IBD patients most likely arises as a result of either exaggerated effector T-cell function or poor regulatory T-cell function, [5]. Neutrophils and monocytes accumulate in the gastrointestinal wall and participate in IBD pathogenesis [6]. Exogenous natural antioxidant enzymes have been used to neutralize ROS or suppress inflammation for IBD treatment which however are limited by low stability, high cost, and potential immunogenicity. [7] .Gold nanoparticles (AuNPs) hold significant promise for treating IBD. They have been widely explored for treating various inflammatory conditions[8]neuroinflammation[9]. Autoimmune inflammation [10], and skin inflammation [11]. Numerous studies have highlighted their efficacy in managing inflammatory bowel diseases [11]. A notable advantage of AuNPs is their exceptional stability, especially at very low pH levels. This stability is crucial for their oral administration, enabling them to reach the intestine where they can exert their therapeutic effects on IBD pathology. This characteristic makes them particularly suitable for targeted interventions in IBD treatment [12]. In this study was

investigated the gastrointestinal prevention effect of Gold nanoparticles against *E. coli* induce ulcer and the immunological effect.

Materials and Methods

Isolation of *E. coli* from a cow's milk with mastitis

A milk samples were collected from cow with clinical mastitis with clinical sign; anomalies, including flakes, blood, pus, or color changes, and the initial milk streams were visually examined in a black-backed strip cup. The teat end was then cleaned with a cotton pad soaked in 70% alcohol, and 15 mL of milk was gathered into a sterile plastic vial after the pre-milking hygiene procedures (stripping, predipping, and drying of teats with paper towels) were completed. Samples were transported to the lab for microbiological culture while being stored in an ice box. Milk samples were collected in the Diyala Governorate between 20 July 2024 and 20 September 2024.

Preparation of the bacteria for induce colitis

The isolated microorganisms were cultivated in a nutrient broth and then incubated for 24 hours to promote growth at a temperature of 37 °C**.** From nutrient broth tube suspension transfers 0.1 mL from the bacterial mixture. Subsequently, spread uniformly over a plate of a Maconkey agar and then it was incubated for a period of 24 hours at 37 °C under aerobic conditions. A single colony was transferred into a test tube holding 5 mL from normal saline. This created a moderate level of turbidity, similar to that of a standard McFarland turbidity solution. The resulting concentration was approximately 10^5cell \ mL.

The housing conditions and animals used in this study

For this study, male rats (Wister albino) were utilized, their ages ranged from 6 to 8 weeks old and arrange from 140-150 grams in weight. The rats were obtained from AL Dowree Centre for Animal Breeding. All rats housed at (18-28°C) and with 12/12 light system. Each rat was put in a box alone for the length of the test. They were provided with chow meal (pellets) and water. The research work was performed with ethical guidelines established by Veterinary Medicine College at the University of Diyala. The Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq, approved this study (Approval no: Vet Medicine (205); August 2024, O and W).

Grouping of laboratory animals according to the requirements of the experiment.

The rats adapted for one week were randomly divided into groups as described below (Abood et al., 2014). All rats fasted for food not for water over night before start experiment and for water two hours before administration Gold nanoparticles and *E.coli* dose to induced ulcer. Normal control group (6 rats): Orally administration distilled water only. Ulcer control group (6 rats): Orally administration distilled water. Omeprazole group (6 rats): Orally administration omeprazole 40mg / Kg. Gold nanoparticles (GNP) G1(6 rats): Orally administration GNP at dose 5 µg/kg. Gold nanoparticles (GNP) G2(6 rats): Orally administration GNP at dose 10 µg/kg. After one hour all rats in groups (except group 1) administration orally *E.coli* 100 µL (10^5 cell / mL). After 2 hours all rats were anesthetized by injecting ketamine and xylazine with dose (0.09 and 0.01 mL/kg), blood samples and colon were collected from each rat.

Gold nanoparticles that used in the study

Sodium citrate-coated rod gold nanoparticles, measuring 38 nm in length and 10 nm in width, were employed in this investigation based on manufacturing Sigma, batch number MKCS7674. Sodium citrate's function is to stop GNPs from aggregating and lessen their toxicity. Furthermore, sodium citrate is not an immunological stimulator in and of itself.

Statistical analysis

The data was analysis by SPSS statistical software. T test and ANOVA test were used to analyze the results. Significant was considered at P value ≤ 0.05.

RESULTS

Out of 50 samples, 20 (40%) were positive for E. coli isolation. Intramammary infection was characterized by the presence of a minimum of 3 colony-forming units of *E. coli*. Milk samples exhibiting more than three distinct colony types were deemed polluted and therefore rejected (NMC, 1999)[13]. The *E. coli* isolates were preserved at −70°C in nutritional broth supplemented with 15% glycerol**.**

Gastrointestinal protective effects of gold nanoparticles

Gastro-protective effect of GNPs was revealed the highly protection from *E. coli* induced ulcer colitis that was showed in the result of this study and presented in the Figure2. The result of gastrointestinal protective effect of GNP against *E.coli* induced ulcerative colitis investigated potential for antioxidant enzyme SOD ng/mL was shown that the level of SOD at group one of gold nanoparticles dose of 5 µg/kg (GNPG1) lower non-significant from ulcer group g at mean ±SD; (2.88± 0.35) ng/mL compere with ulcer group the group two gold nanoparticle dose of 10 µg/kg s(GNPG2) and omeprazole group(OMP) were lower significant at $p \leq 0.05$ .was presented in Figure (3).The result of gastrointestinal protective effect of GNP against *E.coli* induced ulcerative or ulcer colitis was shown that; the Malondialdehyde nmol\mL as oxidative stress marker was shown that the level of gold nanoparticles group one dose of 5 µg/kg (GNP G1) and gold nanoparticles group two dose of 10 µg/kg (GNPG2) are lower than the group ulcer a significant at $p \leq 0.05$. And gold nanoparticles group one dose of 5 µg/kg (GNPG1) was lower non-significant than OMP group at mean± SD(1.49 ±0.35) nmol\mL and), gold nanoparticles group two dose of 10 µg/kg (GNPG2) was lower non-significant than OMP group at mean± SD(1.52±0.27) nmol\mL. Six rat \group .represented in Figure (4).The result of gastrointestinal protective effect of GNP investigated the Tumor Necrosis Factor alpha(TNF-alpha ng\L)was presented in the Figure (5) .Compered with the U control group the level of gold nanoparticles group one dose of 5 µg/kg (GNPG1), GNPG2and OMP groups were lower significant at $p \leq 0.05$. The level of GNPG1 group was lower than the OMP group at mean±SD(241.65±43.49)ng\L and the level of GNPG2 group was lower than the OMP group at mean ±SD(244.12±31.39)ng\L.Six rat \group. Presented in Figure (5).The result gastrointestinal protective effect of GNP through the level of transforming growth factor (TGF-β ng\L) was shown that the gold nanoparticles group one dose of 5 µg/kg (GNPG1)group, gold nanoparticles group two dose of 10 µg/kg (GNPG2) and omeprazole group(OMP) were significant lower than control ulcer group at $p \leq 0.05$. Six rat/group. Was presenter in the Figure (6).Result of gastrointestinal protective effect of GNP : level of prostaglandine E2(ng\mL)in the studied groups : was shown that the gold nanoparticles group one dose of 5 µg/kg (GNPG1)group was lower significant from U control and OMP groups at $p \leq 0.05$. While the gold nanoparticles group two dose of 10 µg/kg (GNPG2)group was lower than the U control group at mean± SD(0.27±0.06) and the the gold nanoparticles group two dose of 10 µg/kg (GNPG2)group was lower than OMP group at mean ±SD(0.27±0.063).compere to U control group the OMP group was lower significant at $p \leq 0.05$,was represented in Figure (7).

DISCUSSION

The condition, mastitis is mean inflammation of udder glandes .Today ,the studies was investigated the common causative agent, *E. coli*, which affect cattle udder glands cause inflammation and have more prevalence than other bacterial agents [14] .newly study (Oleiwi1 andAbood1,2024) was shown that E coli cause clinical mastitis in cow and have Bundle Forming Pilus Gene for Escherichia coli. In this study was include 50 milk samples of clinical mastitis that appear 40% of samples contaminated with *E coli* while 60%infected with other pathogens that shown in table (1).Some intestinal bacterial phylae, such as Firmicutes and Bacteroidetes,have less prevalence compere to other such as Proteobacteria. E. coli present as commensal or pathogenic as enteropathogenic and extraintestinal pathogenic E.coli, these depend on the virulence genes that acquired e.g adhesins and a-hemolysin that link to IBD pathogenesis through damaging intestinal epithelial cell barrier[15].In this study we induce colitis in rat by administration of *E coli* 10^5cell\mL orally one dose to investigate about the gastrointestinal protective effect of gold nanoparticles was shown in Firue(1) Gold nanoparticles with good physiological stability and biosafety that can be noninvasively monitored in vivo by clinical CT after oral administration. Due to its ability to remove intracellular superfluous ROS, upregulate the expression level of antioxidant enzymes and inhibit proinflammatory cytokines, Au 25 NCs have been demonstrated to have good preventive and therapeutic effects on dextran sulfate sodium induced colitis in mice without obvious adverse side effects.[16] .Previous studies suggest that the NPs possess the ability to applications as antibacterial compounds. The antibacterial effects and mechanism of action for NPs were dependent upon composition and surface modifications. [17]. a newly study by(Fontes et al, 2024) they were used algae extract and gold nanoparticles as protective pretreatment for colitis induced by acetic acid in mice a dose of 25 and 50 mg\kg, macroscopically they found a protective effect of algae extract and gold nanoparticles to the colon

compere to colitis group and it similar to that of stander treatment by dexamethasone[12].Recently study by (Fontes et al, 2024) that used Ericaria selaginoides Extract and Gold Nanoparticles as protective treatment in mice induced colitis by acetic acid, they investigate about reduced glutathione levels of colon tissue, the result was found significant decrease the level of reduced glutathione in acetic acid group compere to control group while the level of reduced glutathione in Ericaria selaginoides Extract and Gold Nanoparticles group was present at normal value[12].In this study the result of gastrointestinal protective gold nanoparticles against E.coli induce ulcer colitis was shown in Figure(3)represent that the level of antioxidant enzyme SOD ng/mL at group one of gold nanoparticles dose of 5 μg/kg (GNPG1) lower non-significant from ulcer group at mean ±SD; (2.88± 0.35) ng/mL compere with ulcer group the group tow gold nanoparticle dose of 10 μg/kg s(GNPG2) and omeprazole group(OMP) were lower significant at $p \leq 0.05$. Recently study by shen et al,2022 research about the anti-inflammatory characteristic of melianodiol on dextran sulfate sodium induced colitis in mice they suggest that melianodiol have anti-inflammatory effect through decrease the level of tumor necrosis factor alpha that is proinflammatory cytokine compere with dextran sulfate sodium induce colitis group and melianodiol believed to have antioxidant properties by the decreasing the level of Malondialdehyde which is oxidative stress marker [18]. Many factors accumulates during ulcerative colitis such as oxidative stress and decrease of the anti-oxidansts consequently lead to lipid peroxidation and release of protein known Malondialdehyde witch have mutagenic properties[19].In this study the result of gastrointestinal protective effect of GNP against *E.coli* induced ulcerative or ulcer colitis was shown that; the level of Malondialdehyde nmol\mL as oxidative stress marker was shown that of gold nanoparticles group one dose of 5 μg/kg (GNP G1) and gold nanoparticles group two dose of 10 μg/kg (GNPG2) were lower than the ulcer group a significant at $p \leq 0.05$. And gold nanoparticles group one dose of 5 μg/kg (GNPG1) was lower non-significant than OMP group at mean± SD(1.49 ±0.35)nmol\mL and), gold nanoparticles group two dose of 10 μg/kg (GNPG2) was lower non-significant than OMP group at mean± SD(1.52±0.27)nmol\mL . Six rat \group .represented in Figure (4). Many studies suggested that inhibition of prosraglandinE2 give anti inflammatory properties but during the ulcerative colitis decease the level of PGE2 lead to increase epithelium susceptibility for ulceration [20]. In this study the result of gastrointestinal protective effect of GNP aginst *E.coli* induced ulcerative or ulcer colitis: level of prostaglandine E2(ng\mL)in the studied groups : was shown that the gold nanoparticles group one dose of 5 μg/kg (GNPG1)group was lower significant from ulcer control and omeprazole groups at $p \leq 0.05$. While the gold nanoparticles group two dose of 10 μg/kg (GNPG2) group was lower than the ulcer control group at mean± SD(0.27±0.06) and the the gold nanoparticles group two dose of 10 μg/kg (GNPG2)group was lower than OMP group at mean ±SD(0.27±0.063).compere to U control group the OMP group was lower significant at $p \leq 0.05$,was represented in Figure (7).many studies were reported that the TNF-alpha was elevated during UC that lead to inflammatory cells infiltration, so the main disorder through UC was imbalance of the mucosal immunity manifested by elevation of proinflammatory cytokines like TNF-alpha [21]. In this study the result of gastrointestinal protective effect of GNP aginst E.coli induced ulcerative or ulcer colitis was shown in the Figure(5)that; the level TNF-alpha of gold nanoparticles group one dose of 5 μg/kg (GNPG1), gold nanoparticles group two dose of 10 μg/kg (GNPG2)and omeprazole40mg groups were lower significant at $p \leq 0.05$ from control ulcer group. In this study the result of gastrointestinal protective effect of GNP aginst *E.coli* induced ulcerative was shown in the Figure (6),the level of(TGF-β ng\L) represent that level of gold nanoparticles group one dose of 5 μg/kg (GNPG1), gold nanoparticles group two dose of 10 μg/kg (GNPG2)and omeprazole40mg groups were lower significant at $p \leq 0.05$ from control ulcer group.

CONCLUSION

Gold nanoparticles has the effective ability of gastroprotective against bacterial induced ulcer through the enhancement antioxidant enzyme and modulation the proinflammatory as TGFb and PGE lead to decreased tissue injury and inflammatory cytokines TNF-alpha.

Conflict of interest: No conflict of interest

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Table 1: shown the present of *E coli* in mastitis milk cow

Milk Sample	E.coli	Percentage %
Positive	20	40 %
negative	30	60 %
Total	50	100 %

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Figure 1: represent the *E coli* colony on the MacConkey agar appear pink colonies lactose fermenter and on the EMB ager the present of characteristic green metallic sheen colonies**.**

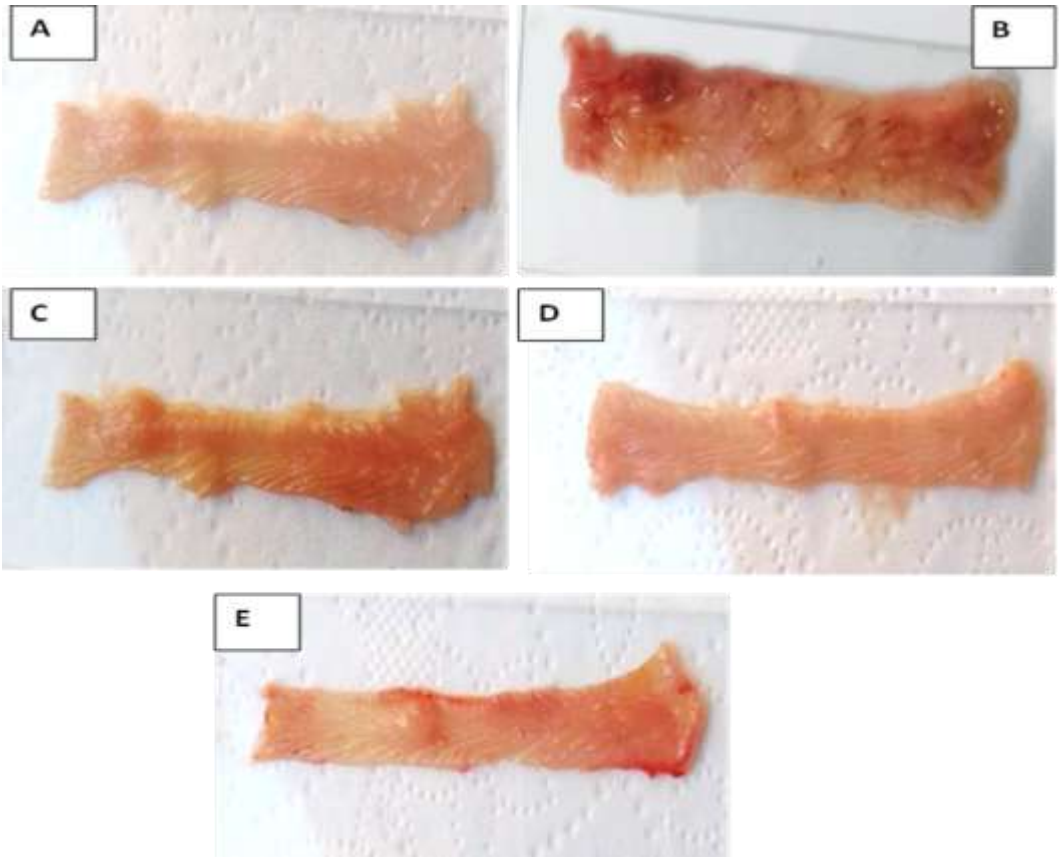

Figure 2: Macroscopically alteration of rat colon after treatment with Gold nanoparticle (GPN) to study the prevention effect from *E. coli* induced colitis. A: Normal rat colon appearance. B: Ulcer group rat was treated with E coli 10^5cell \ml only, black arrow shown extensive ulceration. C: Rat colon administrated of GNP dose of 5 µg/kg *&* *E coli* 10^5cell \ml, the blue arrow shown erosion of the colon lining. D: Rat colon administrated GNP dose of 10 µg/kg & *E coli* 10^5cell \ml, normal colon lining. E: Rat colon administrated omeprazole 40 mg/kg & *E coli* 10^5cell \ml, black arrow shown extensive ulceration.

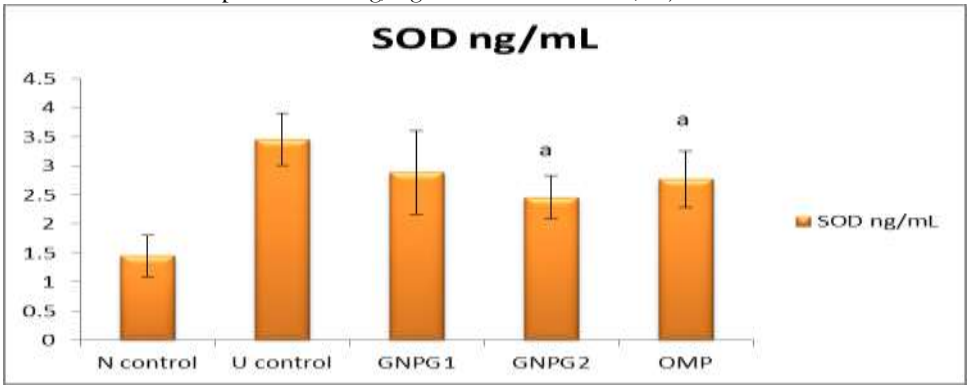

Figure 3 : Presented the result of level of super oxide dismutase (SOD ng/mL) in study groups : Normal group(N control), ulcer control group, group one of gold nanoparticles dose of 5 µg/kg (GNPG1), group tow of gold nanoparticles dose of 10 µg/kg (GNPG2)and omeprazole group(OMP). [a]Significant at $p \leq 0.05$.Six rat\ group.

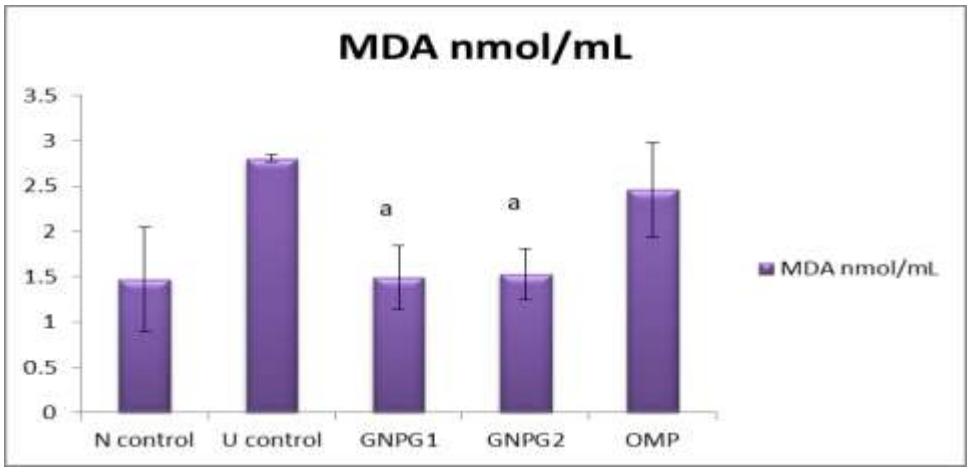

Figure 4: represent the level of Malondialdehyde(MDA nmol\mL).) in study groups : control normal(N control),control ulcer (U control),gold nanoparticles group one dose of 5 µg/kg (GNPG1), gold nanoparticles group two dose of 10 µg/kg (GNPG2) and omeprazole group(OMP) .[a] Significant at $p \leq 0.05$.Six rat \group

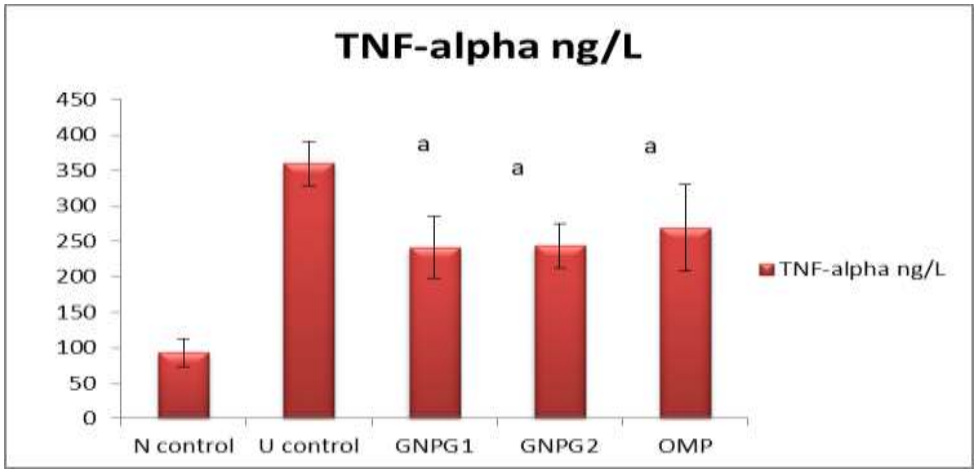

Figure 5: shown level of TNF –alpha in the studied groups : control normal(N control),control ulcer (U control),gold nanoparticles group one dose of 5 µg/kg (GNPG1), gold nanoparticles group two dose of 10 µg/kg (GNPG2) and omeprazole group(OMP) .[a] Significant at $p \leq 0.05$.six rat \group

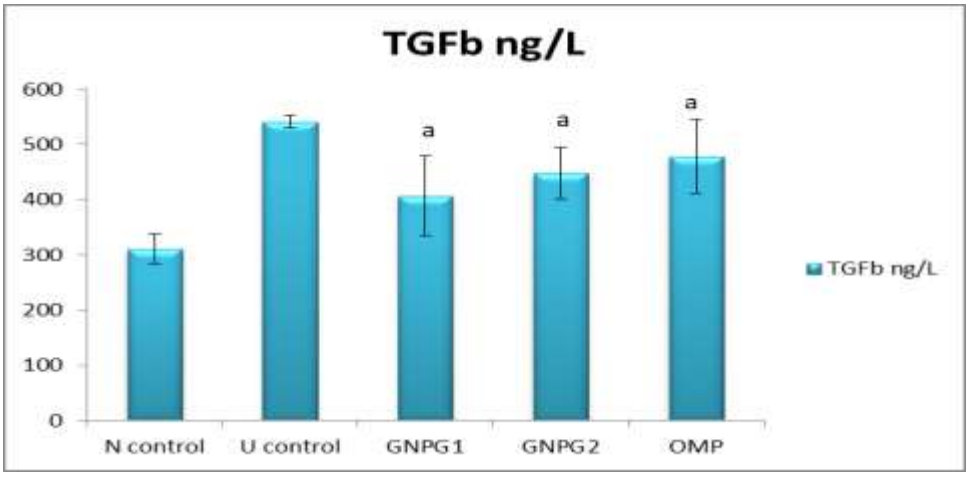

Figure6: show the level of transforming growth factor (TGF-β ng\L).In studied groups: Control normal (N control), control ulcer (U control),gold nanoparticles group one dose of 5 µg/kg (GNPG1), gold nanoparticles group two dose of 10 µg/kg (GNPG2) and omeprazole group(OMP) .[a] Significant at $p \leq 0.05$.six rat \group

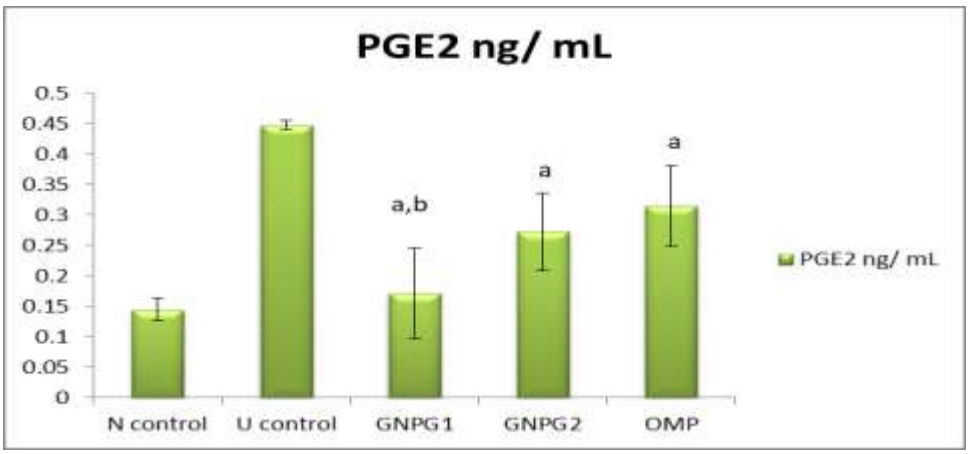

Figure 7: Represent level of prostaglandine E2(ng\mL)in the studied groups:(N control),ulcer group (U control), gold nanoparticles group one dose of 5 µg/kg (GNPG1),gold nanoparticles group two dose of 10 µg/kg (GNPG2)group and omeprazole group(OMP).[a]Significant from U control at $p \leq 0.05$. ,[b]significant from OMP group at $p \leq 0.05$. Six rat \group