

Role of Vitamin B17 against Colitis Bearing Female Mice Induced Variations in Some Blood Parameters

ABSTRACT

Colorectal cancer is also the third most common cause of cancer-related death, and in the US, it ranks as the third most prevalent kind of cancer. The cells that produce mucus to lubricate the colon and rectum can be the source of colorectal cancer. Natural substances known as phytochemicals contain anti-oxidant, anti-inflammatory, and anti-tumor properties. These properties may be crucial for the treatment of a number of debilitating illnesses. The extract of vitamin B17, or amygdalin, is made from the kernels of apricots. Nitrilosides are naturally occurring compounds that contain cyanide. Natural substances called amygdalin have been utilized in traditional Chinese medicine to treat a wide range of illnesses. Its various pharmacological qualities include anti-inflammatory, antioxidant, antitussive, anti-asthmatic, and anti-ulcer effects. findings, When colitis mice were given acetic acid injections via the anal route, they had diarrhoea, obvious rectal bleeding, and weight loss. serum ALT, AST, and ALP values were higher in the colitis group than in the control and vitamin B17 groups. Conversely, there was a notable drop in the colitis group's blood albumin and total protein levels when compared to the control and vitamin B17 groups. In contrast, as compared to the colitis group, vitamin B17 therapy for colitis resulted in a substantial decrease in the levels of ALT, AST, and ALP and an increase in the levels of albumin and total proteins. Serum urea and creatinine levels were higher in the colitis group in comparison to the vitamin B17 and control groups. However, there is a significant decrease in serum urea and creatinine levels in treated colitis with vitamin B17 group when compared with the colitis group. Compared to the vitamin B17 and control groups, the colitis group had higher blood levels of potassium and sodium ions. On the other hand, compared to the colitis group, the treated colitis with vitamin B17 group had significantly lower blood levels of potassium and sodium ions. When compared to the control and vitamin B17 groups, the colitis group's RBC levels and HB% were considerably lower; however, vitamin B17 therapy for colitis regulates and improves these alterations. In contrast to the control and vitamin B17 groups, the colitis group's WBC and platelet counts considerably increased. Conversely, the colitis group's WBC and platelet counts fell when treated with vitamin B17.

Keywords: Colorectal cancer; vitamin B17; mice; liver functions.

1. INTRODUCTION

Cancer is referred to as abnormal cell proliferation that displays a vast array of related syndromes. As the most serious worldwide cause of death, along with an average estimate of 12.7 million cases in 2008, It is Anticipated That 27 million will be new cases identified in 2030. Moreover, roughly 47% of instances of Cancer and 55% of its mortality happen in

underdeveloped parts of the globe [1]. Although continual improvement in the treatment of many tumors, cancer remains creating fear and trouble inlives of suffered patients and in turn their families. Further more, Cancer sufferers usually endure from a measure able degree of emotional anguish [2]. Colorectal cancer (CRCs) is one of the commonhuman malignant neoplasmsand it is the 2nd most commoncancer in large intestine that is consideredthe main public health

problem worldwide [3]. Usually, there is a direct exposure of the rectum to a more concentrated fecal substance. Furthermore, alkaline mucus coats undigested materials passing through the colon. Variations in the colon's and the rectum's pH levels may also affect how susceptible an individual is to outside influences [4]. Rectal cancers are the 2nd most common cancers (28%) in the large intestine after proximal colon cancers (42%) [3]. Therefore, rectal cancers have constantly been considered as a part of CRCs in correlated epidemiological studies. CRC, as one of the main public health problems, is the 3rd most prevalent tumor in men and the 2nd in women worldwide, with a lifetime probability of 4.7-5% [5]. Colitis is a long-term illness that influences people with changes in age where inflammatory agents and free radicals have an important role in its induction. Ulcerative colitis has a combined prevalence of 150-250/100000 population [6]. Anti-inflammatory drugs and steroids are used in the therapy of colitis to reduce inflammation and eventually cure it so that the colon may resume its normal function [7]. Antioxidants are defined as substances that exist naturally in plants and animals which can protect cells from the damaging effects of free radicals [8]. Natural products are defined as a group of biologically active biochemical produced by plants and microorganisms. More than 40% of therapeutic drugs were produced based on natural products and their derivatives in the past decades [9]. Vitamin B17, previously known as laetrile, amygdalin is one among several nitrile sides which natural compounds that naturally contain cyanide are widely found in the seeds of the Prunasins family, which includes Apricots, Apples, Almonds, Peaches, and other Rosaceous vegetation. Among the several species of Prunasins family, Armeniaca semen has been used for the management of pain, leprosy, emphysema, a bronchi colorectal cancer, and asthma and leucoderma [10,11]. Amygdalin consists of two Glucose Molecules, one of which is benzo aldehyde which has an analgesic effect; Hydrocyanic Acid is the second is an anti-neoplastic compound. Apart from all the indicators above, amygdalin has been used to alleviate pain and cure cancers [12].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemical and reagent

Acetic acid: We bought our acetic acid from sigma chemical Co. (St. Louis, Mo., USA).

Olive oil: Olive oil has been bought from sigma chemical Co. (St. Louis, Mo., USA).

Vitamin B17: was bought for the Natural Oils Company on Amazon.

2.1.2 Animals

A total of 40 female Swiss albino mice weighing 20–25 g derived from an Animal house colony Egypt Vaccine Company, Giza, Egypt. The creatures were randomly assigned to rooms with ambient room-temperature at 22 - 25 °C and circumstances of relative humidity a 12-h light/12-h dark cycle, for two weeks, a commercial diet and unlimited water were given to the subjects.

2.1.3 Induction of ulcerative colitis

Using a gavage intrarectally (IR) injection, mice (n =40) were given 100 µl of 2% acetic acid together with 5 µl of olive oil (acetic acid/olive oil mix) every day for a week. To stop fluid leaking, the mice were held horizontally for two minutes right after treatment. After 30 seconds of keeping acetic acid in the colon, the fluid was removed.

2.1.4 Experimental design and animal groups

Equal numbers of mice were separated into four groups (10 mice in each):

Group 1: Control group which only administered intrarectal (IR) injections to the mice 5 µl of olive oil.

Group 2: The mice in this particular group were given Vitamin B17 (175 mg/Kg body weight/ day) (Sigma chemical Co, Germany), given for two weeks orally through a stomach tube, as per [13].

Group 3: Colitis group for a week, mice with induced ulcerative colitis received an intrarectal injection of 100 µl of 2% acetic acid along with 5 µl of olive oil.

Group 4: Post treated group. induction of ulcerative colitis in mice for a week, after which was treated with Vitamin B17 for another 2 weeks.

2.1.5 Sample collection

When the experiment came to a conclusion, the mice that had fasted all night were put to sleep, and two portions of their ocular blood were taken. To measure, the first portion was gathered into a heparinized tube and thoroughly mixed to avoid clot formation. complete blood pictures (CBC), blood specimens.

The second part was centrifuged at 3000 r.p.m for 10 min at room temperature; serum was divided and stored until the assay at -20oC in a clean stopper vial, serum was utilized for kidney functions, and electrolytes.

The colon to be studied by immunohistochemistry and histopathology; three specimens were fixed using a 10 percentage of neutral buffer formalin buffer for a maximum of 48 hours, while the rest were cut and homogenised (Potter-Elvehjem homogeniser) separately in ice-cold buffer (1.15% KCl/0.01mol/l sodium potassium phosphate, pH 7.4). After centrifugation (10,000 g for 20 min at 4 degrees C), the retrieved superior was frozen at -80 degrees for later Biochemical analysis.

2.2 Methods

2.2.1 Complete Blood Counts (CBC)

A laboratory specialist took two centiliters of the patients' blood from their hind vein while they were at work. The blood samples were moved to CBC tubes with EDTA as the anticoagulant, stored in a cold box with ice packs, and then sent straight to the lab for analysis by earlier study [14].

2.2.2 Biochemical assays

The activity of AST and ALT and ALP were determined and Serum albumin was calculated using the suggested methodology, and total protein concentration was calculated using the suggested previous methodology [15]. Serum urea and creatinine concentration were measured using a commercial kit that Diamond, an Egyptian company, provided according to [16]. Correspondingly, Serum sodium and potassium levels were tested by the colorimetric technique using a commercial kit that was supplied by DIATEK business, Egypt according to [17].

2.3 Statistical Analysis

The Statistical Package for the Social Sciences (SPSS software version 16) was used to conduct the analysis. The data were evaluated statistically using one-way ANOVA (Analysis of Variance) and the Least Significant Difference (LSD) tests. The data were provided as the mean± standard error of mean (SEM). Statistical significance was defined as significance at P<0.05. LSD comparisons were used to evaluate the importance of group differences.

3. RESULTS AND DISCUSSION

3.1 Toxicity and Clinical Symptoms

The dose of vitamin B17 did not initiate any side effects for the experimental animals. Our study was conducted to optimize the acetic acid-induced colitis model in the presence of olive oil, and to evaluate the role of endogenous and exogenous stem cell treatment of colitis.

3.2 Changes in Liver Enzymes

Data are expressed as mean ± S.E.M of 8 observations. Where G1, control; G2, vitamin B17; G3, colitis; G4, treated colitis with vitamin B17. (a) significant difference compared to the control group. (b) Significant difference compared to the colitis group.

3.3 Kidney Functions and electrolytes

3.3.1 Changes in kidney functions (urea , creatinine) and Serum electrolytes (Na , K) levels in In the fourth group and compared to the third and first group, and the second group and compared to the first group.

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Fig. 1. Anal injections of acetic acid-induced colitis(arrow) in mouse

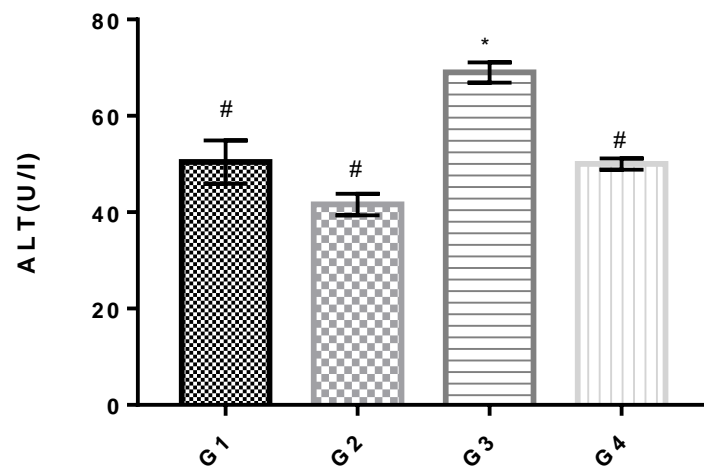


Fig. 2. Changes in the levels of aspartate aminotransferase [ALT] in study groups

The mean \pm S.E.M. of eight observations is used to express the data. where G1, control; G2, vitamin B17; G3, colitis; and G4, vitamin B17-treated colitis. (*) noteworthy variation in relation to the control group. (#) Significant difference compared to the colitis group

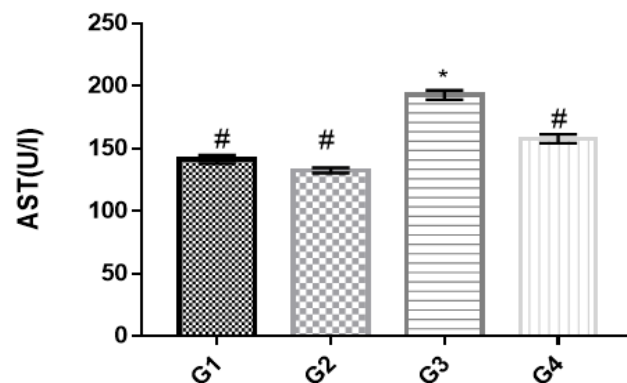


Fig. 3. Changes in the levels of alanine aminotransferase [AST] research teams

The mean \pm S.E.M. of eight observations is used to express the data. where G1, control; G2, vitamin B17; G3, colitis; and G4, vitamin B17-treated colitis. (*) noteworthy variation in relation to the control group. (#) Significant difference compared to the colitis group

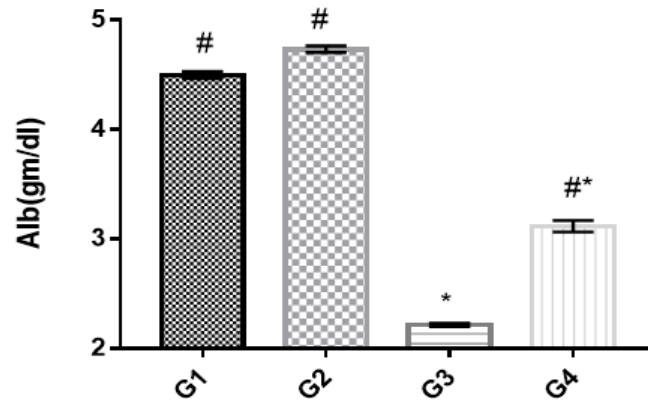


Fig. 4. Changes in the levels of albumin [Alb] in study groups

The mean ± S.E.M. of eight observations is used to express the data. where G1, control; G2, vitamin B17; G3, colitis; and G4, vitamin B17-treated colitis. (*) noteworthy variation in relation to the control group. (#) Significant difference compared to the colitis group

Table 1. Changes in the levels of liver functions in study groups

	ALT (U/l)	AST (U/l)	Alb (gm/dl)	ALP (U/l)	Total protein (g/dl)
G1	48.5 ^b ± 3.15	140.5 ^b ± 4.13	4.55 ^b ± 0.07	125.1 ^b ± 4.25	5.74 ^b ± 0.37
G2	40.9 ^b ± 2.85	134.1 ^b ± 3.50	4.76 ^b ± 0.09	117.7 ^b ± 4.11	6.06 ^b ± 0.19
G3	68.5 ^a ± 1.87	192.2 ^a ± 5.18	2.23 ^a ± 0.10	169.0 ^a ± 7.86	4.49 ^a ± 0.35
G4	49.5 ^b ± 1.40	157.0 ^{ab} ± 4.68	3.04 ^b ± 0.19	130.8 ^{ab} ± 8.53	6.458 ^b ± 0.29

Table 2. Changes in the levels of kidney functions and electrolytes in study groups

	Urea (mg/dL)	Creat (mg/dL)	Na+ (mmol/L)	K+ (mmol/L)
G1	24.0 ^b ± 1.03	0.43 ^b ± 0.03	135.3 ^b ± 4.20	5.29 ^b ± 0.18
G2	23.2 ^b ± 1.26	0.40 ^b ± 0.03	135.9 ^b ± 3.39	5.29 ^b ± 0.09
G3	36.5 ^a ± 2.88	0.83 ^a ± 0.05	144.0 ^a ± 7.85	1.20 ^a ± 0.22
G4	30.7 ^{ab} ± 2.15	0.62 ^b ± 0.04	137.9 ^b ± 6.55	5.75 ^{ab} ± 0.09

The mean ± S.E.M. of eight observations is used to express the data. where G1, control; G2, vitamin B17; G3, colitis; and G4, vitamin B17-treated colitis. (a) notable distinction in comparison to the control group. (b) very little variation from the group with colitis

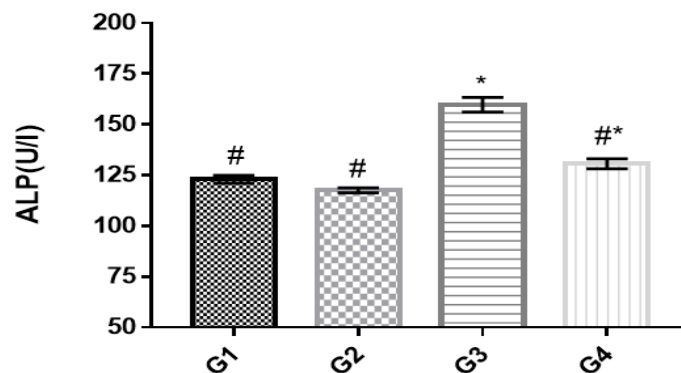


Fig. 5. Changes in the levels of alkaline phosphatase [ALP] in studies groups

The data is presented as the mean ± S.E.M. of eight observations. As follows: G1, control; G2, vitamin B17; G3, colitis; G4, vitamin B17-treated colitis. * Significant variation from the control group. (#) Significant difference compared to the colitis group

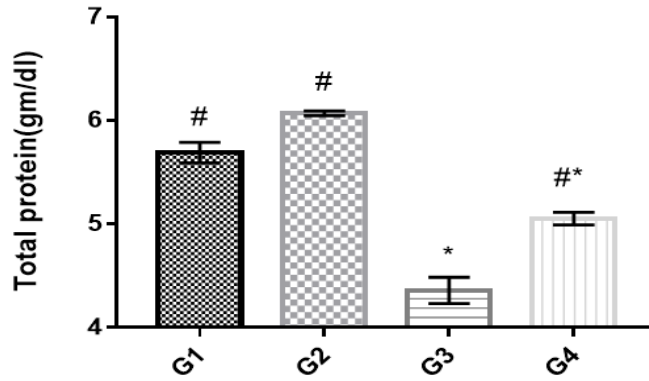


Fig. 6. Changes in the levels of liver functions (total proteins) in studies groups
 The mean \pm S.E.M. of eight observations is the data's expression. in which G1, control; G2, vitamin B17; G3, colitis; and G4, vitamin B17-treated colitis. (*) noteworthy variation from the control group. (#) Significant difference compared to the colitis group

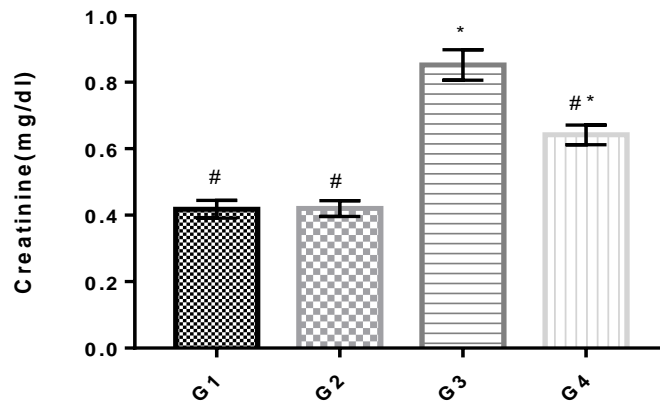


Fig. 7. Changes in the levels of kidney functions (creatinine) in studies groups
 Data are expressed as mean \pm S.E.M of 8 observations. Where G1, control; G2, vitamin B17; G3, colitis; G4, treated colitis with vitamin B17. (*) significant difference compared to control group. (#) highly non-significant difference compared to the colitis group

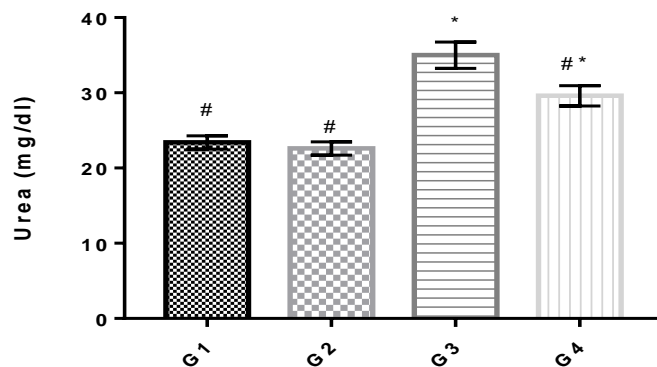


Fig. 8. Changes in the levels of kidney functions (urea) in study groups
 Data are expressed as mean \pm S.E.M of 8 observations. Where G1, control; G2, vitamin B17; G3, colitis; G4, treated colitis with vitamin B17. (*) significant difference compared to control group. (#) Significant difference compared to the colitis group

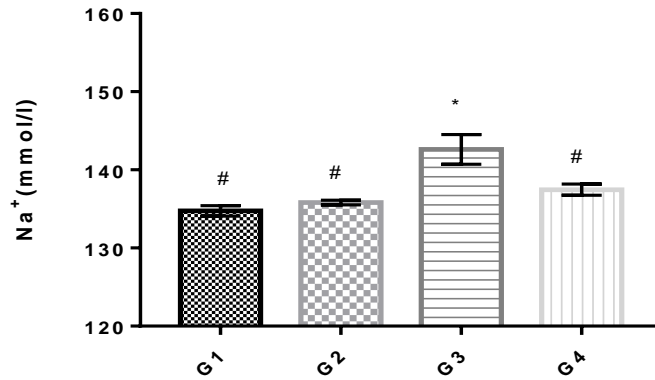


Fig. 9. Changes in the levels of serum electrolytes (sodium ions) in study groups
 Data are expressed as mean \pm S.E.M of 8 observations. Where G1, control; G2, vitamin B17; G3, colitis; G4, treated colitis with vitamin B17. (*) significant difference compared to control group. (#) Significant difference compared to the colitis group

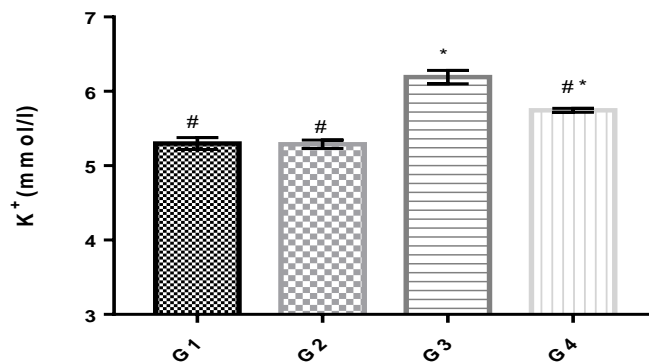


Fig. 10. Changes in the levels of serum electrolytes (potassium ions) in study groups
 The mean \pm S.E.M. of eight observations is used to express the data. where G1, control; G2, vitamin B17; G3, colitis; and G4, vitamin B17-treated colitis. (*) noteworthy variation in relation to the control group. (#) Significant difference compared to the colitis group

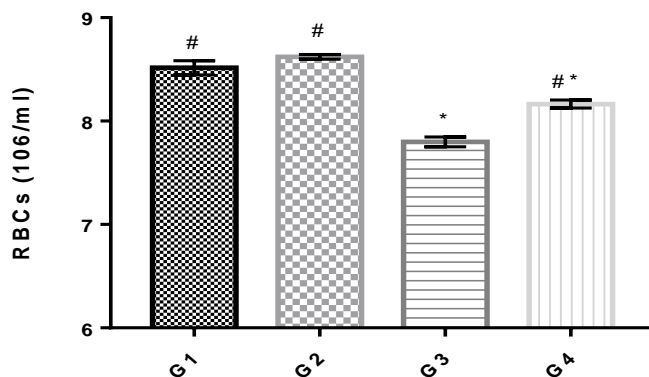


Fig. 11. Changes in the RBCs in the study groups
 Data are expressed as mean \pm S.E.M of 8 observations. Where G1, control; G2, vitamin B17; G3, colitis; G4, treated colitis with vitamin B17. (*) significant difference compared to control group. (#) highly non-significant difference compared to the colitis group

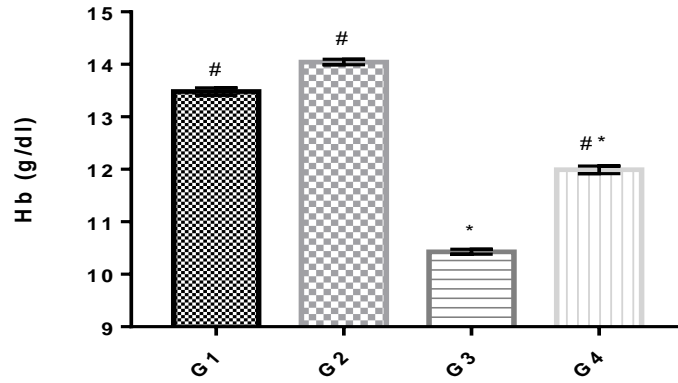


Fig. 12. Changes in the Hb in the study groups

The mean \pm S.E.M. of eight observations is used to express the data. where G1, control; G2, vitamin B17; G3, colitis; and G4, vitamin B17-treated colitis. (*) significant difference compared to control group. (#) highly non-significant difference compared to the colitis group

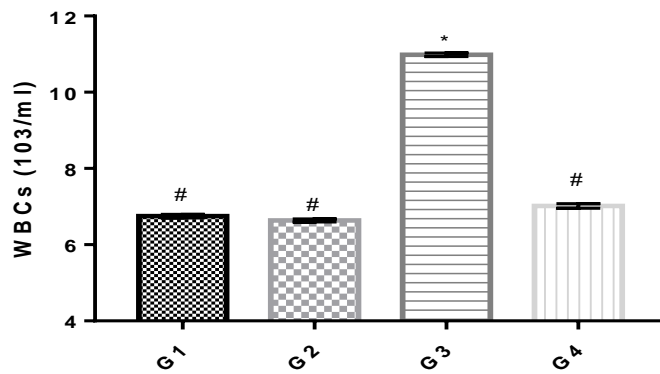


Fig. 13. Changes in the WBCs in the study groups

The mean \pm S.E.M. of eight observations is used to express the data. where G1, control; G2, vitamin B17; G3, colitis; and G4, vitamin B17-treated colitis. (*) significant difference compared to control group. (#) highly non-significant difference compared to the colitis group

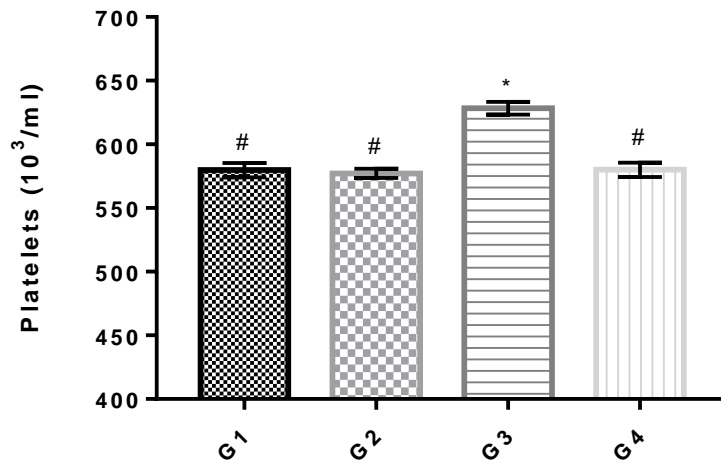


Fig. 14. Changes in the platelets counts in the study groups

Data are expressed as mean \pm S.E.M of 8 observations. Where G1, control; G2, vitamin B17; G3, colitis; G4, treated colitis with vitamin B17. (*) significant difference compared to the control group. (#) highly non-significant difference compared to the colitis group

Table 3. Changes in RBCs, Hb%, WBCs levels and platelets counts in different groups under study

	RBCs (10⁶/ml)	Hb (g/dL)	WBCs (10³/ml)	Platelets (10³/ml)
G1	8.59 ^b \pm 0.35	13.50 ^b \pm 1.07	6.75 ^b \pm 0.36	582 ^b \pm 18.1
G2	8.62 ^b \pm 0.42	14.06 ^b \pm 0.86	6.68 ^b \pm 0.29	577 ^b \pm 12.2
G3	7.80 ^a \pm 0.29	10.48 ^a \pm 0.53	11.06 ^a \pm 0.92	627 ^a \pm 21.6
G4	8.16 ^{ab} \pm 0.48	11.92 ^{ab} \pm 1.05	6.91 ^b \pm 0.55	582 ^b \pm 20.5

The mean \pm S.E.M. of eight observations is used to express the data. where G1, control; G2, vitamin B17; G3, colitis; and G4, vitamin B17-treated colitis. (a) notable distinction in comparison to the control group. (b) very little variation from the group with colitis

3.4 The Changes Blood Parameters in Different Groups

The amount of vitamin B17 did not initiate any side effects for the experimental animals. Our study was conducted in order to maximize the Model of colitis caused by acetic acid in the presence of oil from olives, and to evaluate the role of endogenous and exogenous stem cell treatment of colitis. Albino CD1 mice were administrated via infrared infusion of acetic acid. Acetic acid and olive oil (1 μ L) were simultaneously administered. Colitis is associated with recurrent inflammatory gastrointestinal tract problems accompanied by diarrhoea, weight loss, nausea, and many clinical characteristics such as inflammatory cell infiltration and a loss of mucosal integrity [18]. These results were agreed with [19], who reported that; around 50% of the Liver metastases will occur in 20% of colorectal cancer patients over their lifetime; these metastases will be synchronous in 30% of cases meta chronous. Acute liver failure is not often caused by malignant liver disease. Malignancy induces liver failure through a complex process that involves both intrahepatic and extrahepatic biliary blockage and a direct decrease in the volume of a healthy, functioning liver [20]. Our results agree with the earlier study and they reported that; colorectal cancer in patients induced liver dysfunction [21]. This is in agreement with the findings of who noted a rise in ALP activations in liver and colorectal cancer. Elevated amounts were also seen in other cancer types in this investigation. This rise in ALP serum activity might be a sign of metastatic illness [22]. The liver's ALT and AST enzymes are responsible for rearranging the protein building components. The injured liver cells release the enzymes. Similar findings were also reported by [23]. Also; [24]. found that a hepatocellular injury may be the cause of the rise in AST and ALT activities,

which may explain why patients with liver cancer have higher levels of these enzymes than patients with other cancers. Also; lower levels of serum albumin are prevalent in patients with advanced cancer, and research from the past suggests that they might increase the risk of death from inflammation and starvation. Advanced cancer patients frequently have lower blood albumin levels, and a prior study found that these may contribute to increase death rates from inflammation and malnourishment [25] As per the report of [26] there was increased in the levels of creatinine is a kidney dysfunction index. In our study; colitis- induced increase in Urea and creatinine levels suggested renal impairment. Maleichydrazide significantly increased blood sample levels of serum urea and creatinine, which had harmful consequences and inhibited the production of proteins and nucleic acids. Maleichydrazide also induced carcinogenic effects in mice and rats [27].

RBC levels and HB% in the colitis group were significantly decreased when compared with In contrast, vitamin B17 therapy for colitis was administered to control and vitamin B17 groups modulate and improved this changes, WBCs levels and platelets counts in the colitis group were significantly increased when compared With comparison to the control and vitamin B17 groups; vitamin B17 therapy for colitis decreased the WBCs levels and platelets counts when compared with colitis group [14].

5. CONCLUSION

The amount of vitamin B17 did not initiate any side effects for the experimental animals. Our study was conducted to maximize the colitis model caused by acetic acid when olive oil is present and to evaluate the role of

endogenous and exogenous stem cell treatment of colitis.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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