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USING OF WHEY PROTEIN AS ANTIBACTERIAL IN YOGURT AND ITS ROLE IN EXPERIMENTALLY INFECTED RATS WITH *ESCHERICHIA COLI* BY EXAMINING HISTOPATHOLOGICAL CHANGES AND SOME BLOOD PARAMETERS

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ABSTRACT

The present work investigated the effects of whey protein on *Escherichia coli* inoculated in yogurt. The results obtained indicated that the addition of whey protein (WP) led to complete inhibition of *E.coli* on the 3rd day at a concentration of 3.13%, while at a concentration of 1.56%, the microorganism required 5 days for complete inhibition. The properties of yogurt were evaluated and the overall acceptability of those fortified with WP was very satisfying, more than the plain ones. 18 male rats were divided into three groups: 1) the control group, 2) the infected group (administered 2 mL of sterile phosphate-buffered saline containing 1×10^9 colony-forming units of *E. coli* per mL on days 7 and 14), and 3) the treated group (which received the same protocol as the *E. coli*-infected animals, supplemented with whey protein at a dose of 2 mL/kg daily from day 7 to day 21, the end of the experiment). WP was investigated the effect of experimental infection with *Escherichia coli* and treatment with whey protein on various biochemical parameters and histopathological changes in rats. At the end of the experiment, blood samples were collected for biochemical analysis. Samples from the liver, kidney, spleen, and intestine were collected for histopathological examination. The obtained results revealed that whey protein supplementation has a strong antibacterial effect and can reduce the severity of *E. coli* infection at 1.56 and 3.13% and the histopathological alterations were observed in different organs.

Key words: whey protein, yogurt, biochemical, histopathology, sensory properties.

INTRODUCTION

Yoghurt is a dairy product that has been fermented with lactic acid bacteria (LAB). It is well-liked by consumers because of its high nutritional content and several health advantages, including enhancing immune regulation, reducing intestinal discomfort, and improving lactose intolerance (Du *et al.*, 2023 and Zhou *et al.* , 2024). But yoghurt is a perfect enrichment medium for the growth of harmful foodborne bacteria like *E. coli*,

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which can enter milk and milk products due to a variety of internal and external factors, including contaminated equipment, poor worker personal hygiene, unsanitary production lines, and contaminated production rooms (El Biaa, 2018).

So, growing customer demand for healthy foods has led to increased research into creating novel fortified yoghurt varieties that are enhanced with grains, fruits, vegetables, plant extracts, and other nutrients (Ahmad *et al.,* 2022 and Brodziak and Krol, 2023).

According to Ahmed and Shimamoto (2015), *E. coli* is a bacterium that frequently clings to raw, unpasteurized meat and dairy products, contaminating food and leading to food-borne diseases. These bacteria's capacity to create biofilms is what makes them so challenging to eradicate. Proteins, polysaccharides, and other substances make up the biofilm matrix, which can shield the bacteria inside and enable environmental adaptation (Vestby *et al.,* 2020). In rats, *E. coli* caused harmful biochemical and histological alterations (Radwan *et al.,* 2021). Different organs sustain varying degrees of damage due to oxidative stress and inflammation caused by *E. coli* (Long *et al.,* 2022).

Whey protein is a naturally occurring by-product of making cheese that is left in solution when milk is curdled with rennet or treated with acid. According to Ramos *et al.* (2015), the primary constituents of bovine whey protein are β-lactoglobulin (β-LG; 35–65%) and α-lactalbumin (α-LA; 12–25%), with a little amount of immune-globulins (8%), bovine serum albumin (BSA; 6%), lactoferrin (LF; <3%), and lactoperoxidase (0.3%). Numerous bioactive peptides found in WP have biological effects include antibacterial, antiinflammatory, and anti-hypertensive properties. These peptides are used as an active element in the creation of functional foods (Shayanti and Sanjeev, 2020).

According to a number of studies, WP may have antioxidant properties since it can raise tissue and blood glutathione (GSH) levels, which in turn raises the scavenger of free radicals (Peng *et al.,* 2009). The biochemical parameters, oxidative stress markers, and histological picture were all improved by the WP treatment (Gad *et al.,* 2011).

So, the objective of this study was to examine the antibacterial effect of whey protein (WP) on *E.coli* bacteria inoculated in yogurt and study its effect on various biochemical parameters and histopathological picture in rats.

MATERIALS AND METHODS

Ethical approval:
The experiment in this study was conducted in accordance with the ethical guidelines for the care and use of animals and approved by the Institutional Animal Care and Use Committee (ARC-IACUC) of the Agricultural Research Center, under approval number ARC-AHRI 16524

Materials:
Whey protein was obtained from limitless ALPHA, dietary supplement. Reg. No. 5958/2023.

Methods:
1- Bacterial suspension preparation:
The bacterial strain *E. coli* (ATCC: 9637) was obtained from Animal Health Research Institute (AHRI, Giza, Egypt) a licensed food facility. It was grown in *E. coli* selection broth in accordance with (BAM, 2022) and then incubated before being inoculated into selective agar. The bacterial suspension was vortexed, compared to a concentration of 0.5 McFarland Standard as per (McFarland, 1907), and then diluted to be justified to 10^5. Pure colonies were then injected into 5 ml of saline, and the antibacterial activity of whey protein against the strain under investigation was assessed.

2- Evaluation of the antibacterial activity of whey protein against bacterial strain (*in vitro*)

Agar well diffusion method was used to test the antibacterial activity of whey protein against *E. coli* (CLSI, 2011). A sterile cotton swab was used to apply a pathogenic strain (0.1 ml of a previously manufactured and tested microbe) to the surface of Muller Hinton agar, which had been placed into petri dishes and left to harden. To saturate the agar with the pathogenic strain, the plates were left to stand at 37°C for two hours. 50 µL of each whey protein concentration (100, 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.8%, respectively) was inoculated straight into a well created with a cup drill (0.5 cm). The plates were incubated for twenty-four hours at 37°C. The zones of inhibition surrounding each well were measured in millimetres following the incubation. The test was performed in triplicate.

3- Anti-bacterial effect of whey protein in manufactured yogurt.

Yogurt was manufactured according to (Ranok *et al.,* 2021) with little modification; low-fat bovine milk (1.0% fat and 8.5% solids non-fat) was heated at 75°C for 15 min, then immediately cooled, and kept at a constant temperature of 42°C. To inoculate the milk, 0.005% (w/v) of the lyophilized yoghurt starter cultures containing a blend of *Lactobacillus delbrueckii subspp bulguricus* and *Streptococcus thermophilus* (YoFlex® Express 2.0 Chr-Hansen, Denmark) were added. Four sets of yoghurt trials were created; one was removed as a positive control using *E. coli* inoculum and thoroughly mixed without the addition of whey protein. Whey protein was added to two jars at concentrations of 1.56 and 3.13%, while the fourth jar served as a control negative and neither *E. coli* nor whey protein was added.

A loopful of the inoculated jars was streaked onto EMB plates at 37ºC for 24-48 hours as time zero experiment, after curdling, and every two days until the experiment ended. The fermentation was stopped when the pH reached 4.6, and the yoghurt was then stored at 42 °C in an incubator and then at 4 °C (in the refrigerator).

4- Organoleptic analysis (Fernandes *et al.,* 2008).

Jars of control yoghurt had been created; these were free of the previous microorganism but inoculated with whey protein at concentrations of 1.56 and 3.13%, respectively. To style the trials, thirty panelists of varying ages and backgrounds were selected. The percentages of scores for taste, color, smell, texture and overall acceptability (OAA) were noted.

Experimental study design:

18 male albino rats weighing 160–180 g and about 6 weeks of age were purchased from Animal house at Faculty of Medicine's animal home. For seven days, the rats were kept in cages with a 12-hour light/dark cycle at a temperature of 22°C. For acclimatization animals get rodent food pellets, water and libidum. The animals were divided into 3 groups: 1) the control group, 2) the infected group (which received 2 mL of sterile phosphate-buffered saline containing 1×10^9 colony-forming units of *E. coli* per mL on days 7 and 14), and 3) the treated group (which was given the same protocol as the *E. coli*-infected groups, but with whey protein added at a dose of 2 mL/kg daily starting on day 7 to day 21, the end of the experiment). At the end of the experiment, blood samples were collected under aseptic conditions in clean, dry centrifuge tubes. The rats were then sacrificed, and their liver, kidney, spleen, and intestine were collected for histopathological examination.

Blood samples:

Using a capillary tube, blood samples were drawn from the retro-orbital venous plexus, transferred to sterile, plain tubes

devoid of anticoagulant, and centrifuged for 15 minutes at 3,000 rpm to get the serum. After that, the serum was kept at -20°C until it was subjected to biochemical analysis.

Biochemical blood analysis:
Albumin (Alb) and serum total protein (STP) were estimated as part of the serum analysis, and globulins (Glob) were measured by deducting Alb values from STP. Additionally, the Alb/Glob ratio was computed. Following the manufacturer's instructions, a semiautomatic biochemical analyzer and commercially available test kits (Biodiagnostic Co., Egypt) were used to measure the levels of alanine amino-transferase (ALT), aspartate aminotrans-ferase (AST), creatinine, and urea. Biochemical investigations were carried out at the Department of Biochemistry, Faculty of Medicine.

Histopathological study:
Samples from liver, kidney, spleen, and small intestine were preserved in 10% neutral buffered formalin for histological analysis. After being cut to a thickness of 4 microns, the paraffin-embedded sections were stained using haematoxylin and eosin (H&E). A light microscope was used to view the histological preparations (Bancroft and Layton, 2013).

Statistical analysis:
For statistical analysis, analysis of variance (ANOVA) was utilized, and groups were compared using nonparametric techniques (Mean ± SEM). Software called GraphPad Prism (San Diego, CA, USA, Version 8) was used to run Kruskal-Wallis nonparametric ANOVA. The threshold for statistical significance was set at $P<0.05$.

RESULTS

Table 1: Minimum inhibitory concen-tration (MIC) of whey protein for inhibition of *E.coli*.

Concentration of whey protein	Mean ±SD
100%	33±1.37
50%	28.35±0.60
25%	19.56±0.70
12.50%	18.33±0.60
6.25%	14.33±0.60
3.13%	11.93±0.40
1.56%	8.2±0.65
0.80%	0±0

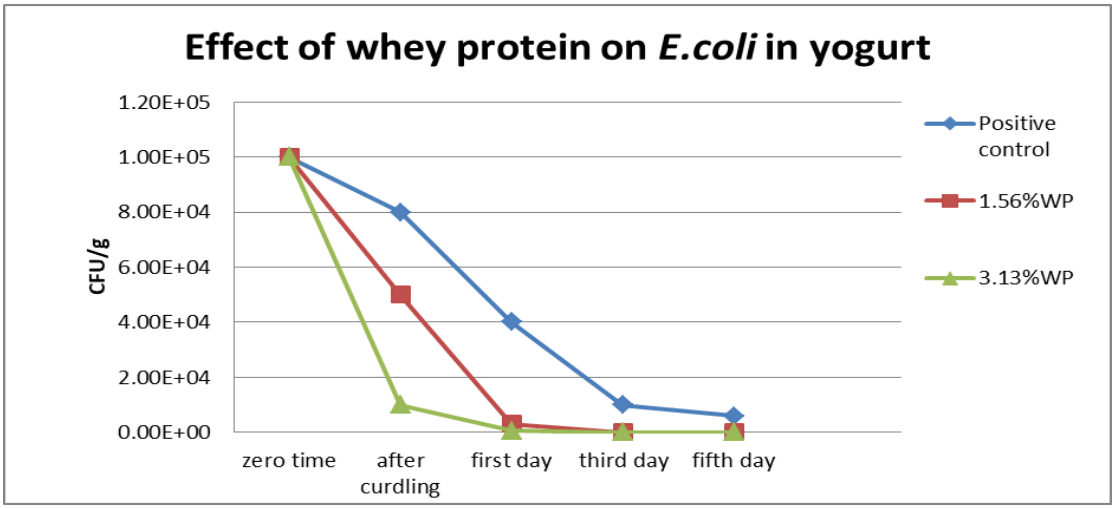

Figure 1: Inhibition of *E.coli* in yogurt after addition of 1.56 and 3.13% of whey protein.

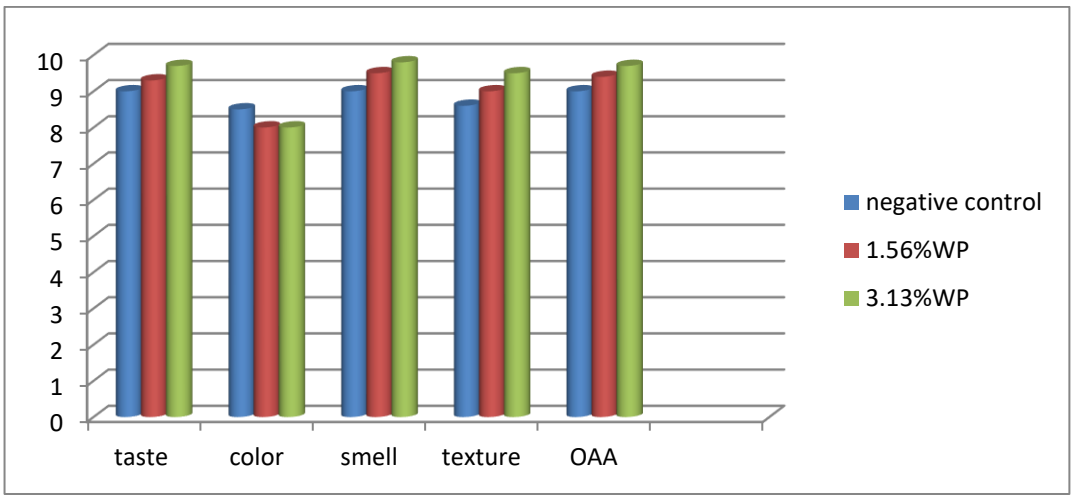

Figure 2: Sensory evaluation of yogurt after addition of 1.56 and 3.13% of whey protein.

Table 2: Liver and kidney function test in serum of the treated groups.

Parameters	Control	Infected	Treated
ALT (U/L)	4.3 ± 0.3 [a]	6.4 ± 0.4 [b]	4.4 ± 0.2 [a]
AST(U/L)	8.0 ± 0.5 [a]	11.6 ± 1.1 [b]	7.5 ± 0.2 [a]
Urea (mg/dL)	22.2 ± 2.1 [a]	35.4 ± 5.9 [a]	42.0 ± 2.7 [a]
Creatinine (mg/dL)	0.4 ± 0.1 [a]	0.9 ± 0.2 [a]	1.1 ± 0.1 [a]

The data expressed in Mean ± SEM. The value considered significant between groups at ($P < 0.05$), expressed with different letters.

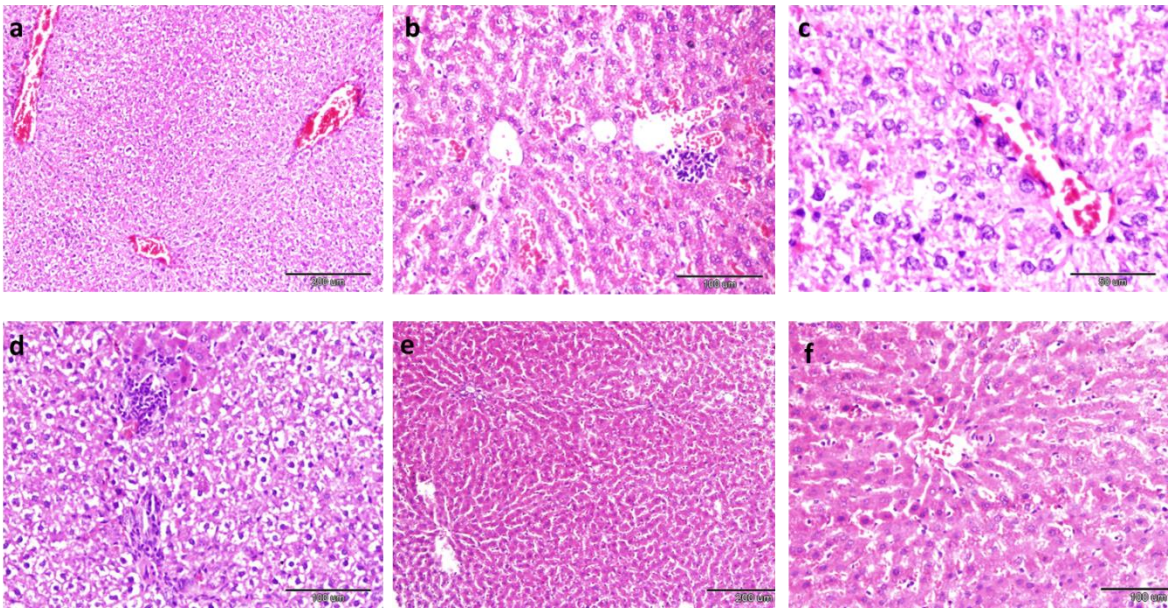

Figure 3: (a) Liver from infected group showed congestion of the central vein (X100). **(b)** Liver from infected group showed dilated hepatic sinusoids, with areas of haemorrhage (X200). **(c)** Liver from infected group showed Centrilobular necrosis with vascular congestion (X400). **(d)** Liver from the infected group showed vacuolar degeneration of hepatocytes and inflammatory cells infiltration (X200). **(e)** Liver from treated group showed restoration of hepatic architecture (X100). **(f)** Liver from treated group showed more or less normal hepatocytes (X200).

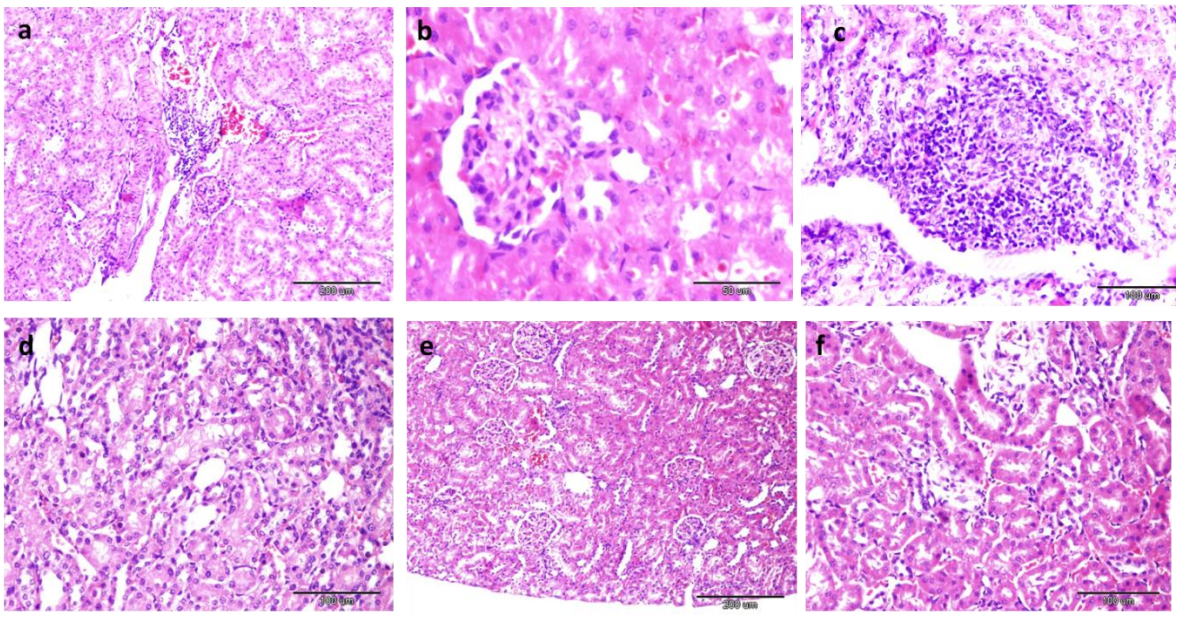

Figure 4: (a) Kidney from the infected group showed hemorrhage in both the renal cortex and medulla (X100). **(b)** Kidney from the infected group showed coagulative necrosis with apoptotic cells (X400). **(c)** Kidney from the infected group showed widespread leukocytic infiltration in the interstitial tissue (X200). **(d)** Kidney from infected group showed renal tubular vacuolization and flattening of tubular epithelium (X200). **(e)** Kidney from the treated group showed nearly normal renal parenchyma (X100). **(f)** Kidney from the treated group showed less severe changes (X200).

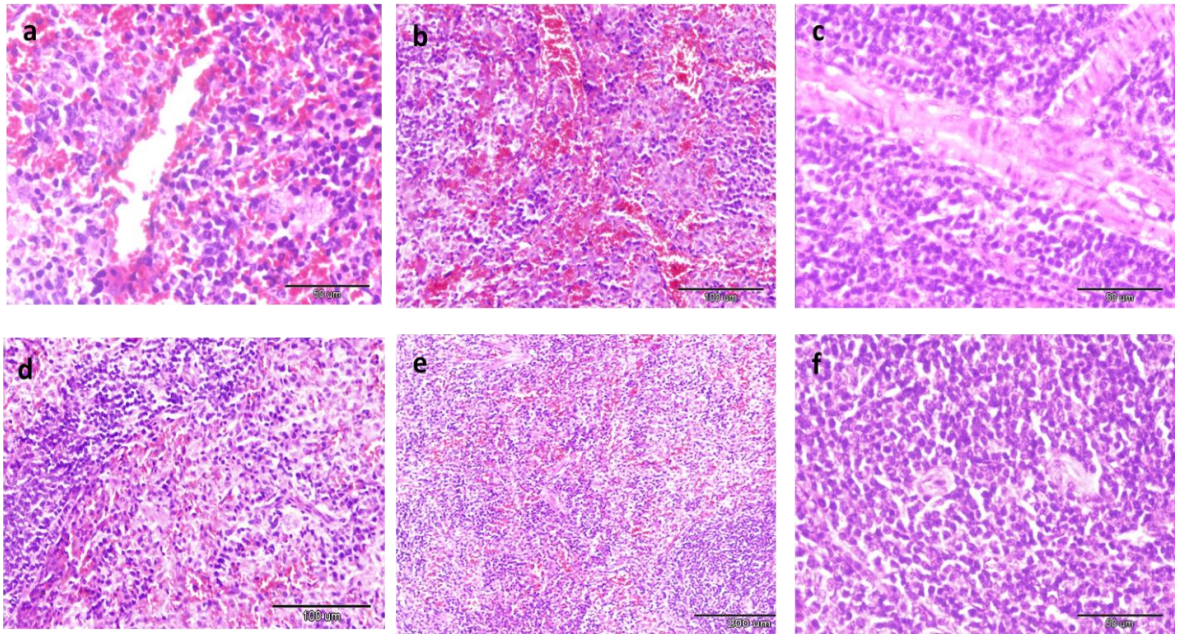

Figure 5: (a) Spleen from infected group showed severe depletion of lymphocytes (X400). **(b)** Spleen from the infected group showed hemorrhage of red pulp with necrobiosis in the center of white pulp (X200). **(c)** Spleen from infected group showed necrobiosis in the arterial wall (X400). **(d)** Spleen from infected group showed leukocytic infiltration (X200). **(e)** Spleen from treated group showed less severe changes (X100). **(f)** Spleen from the treated group showed preservation of splenic architecture (X400).

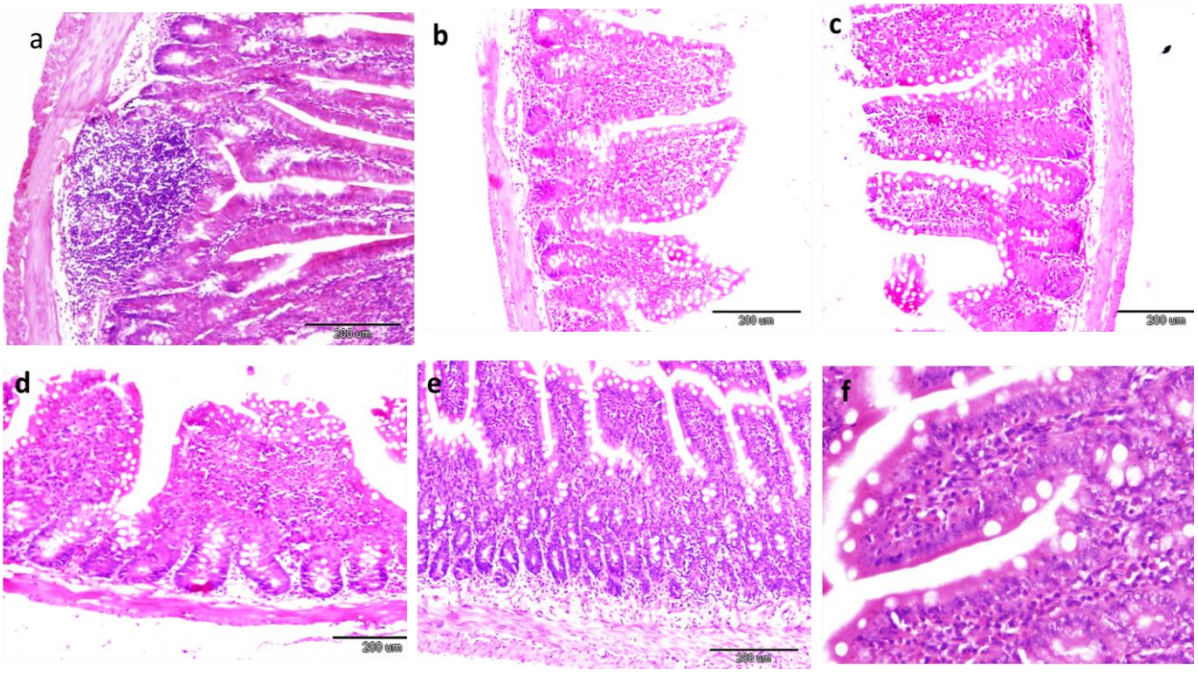

Figure 6: (a) Intestine from infected group showed inflammatory cell infiltration in the lamina propria (X100). (b) Intestine from the infected group showed goblet cell hyperplasia (X100). (c) Intestine from infected group showed sloughing of the upper tips of some intestinal villi (X100). (d) Intestine from the infected group showed thickening of intestinal villi with hyperplasia of intestinal gland lining epithelium (X100). (e) Intestine from treated group showed less severe changes (X100). (f) Intestine from the treated group showed nearly normal epithelial lining (X200).

Table 3: Proteinogram in serum of the treated groups.

Parameters	control	Infected	Treated
Total protein (TP) (g/dL)	5.9 ± 0.1 [a]	4.2 ± 0.2 [b]	6.2 ± 0.4 [a]
Albumin (g/dL)	3.2 ± 0.1 [a]	2.4 ± 0.2 [b]	3.5 ± 0.2 [a]
Globulin (g/dL)	2.7 ± 0.1 [a]	1.8 ± 0.2 [b]	2.7 ± 0.4 [a]
A/G ratio	1.2 ± 0.1 [a]	1.3 ± 0.1 [a]	1.3 ± 0.1 [a]

The data expressed in Mean ± SEM. The value considered significant between groups at ($P < 0.05$), expressed with different superscripts.

DISCUSSION

Due to its nutritional profile, yoghurt is a nutrient-dense food with a variety of health benefits, making it one of the most popular fermented dairy products worldwide (Rajvir *et al.*, 2021). There is growing evidence that milk and fermented milk products are good for the body (Aryana and Olson, 2017; Donovan and Hutkins, 2018). However, because of their high nutritional value, some fermented milk products, such as yoghurt, are prone to microbial infection, especially when left at room temperature. This compromises the product's quality over time and changes its flavor and smell so, using natural and appropriate antimicrobial agents to maintain yogurt's quality and prolong its shelf life is crucial because this is a major concern in the dairy industry (Karimi *et al.*, 2021). As a common food item contaminant and a reliable indicator of faecal pollution, *Escherichia coli's* presence in dairy products may be a sign of contamination with other enteropathogenic

microorganisms that pose a risk to public health, particularly Shiga toxin-producing *E. coli* (STEC), which can be spread to humans through contaminated food. Thus, cleanliness and efficient control measures are the primary factors influencing the prevalence of STEC in raw dairy products (Velázquez-Ordoñez *et al.,* 2019). According to Tamime and Robinson (2007), research and development in dairy factories has been continuously creating different types of fortified yoghurt by adding health-promoting ingredients like probiotic bacteria, vitamin D, essential amino acids, and whey protein-an ingredient that has drawn a lot of attention in the dairy manufacturing industries due to its high nutrient value.

Table (1) indicated a strong positive correlation between whey protein concentration and the measured variable. Higher concentrations of whey protein result in higher values for the variable, which gradually diminishes as the concentration decreases. This trend suggests that the measured property is highly dependent on whey protein concentration and may be proportional up to a certain threshold. The reduction in standard deviation at intermediate concentrations (e.g., 50% and 3.13%) indicates improved consistency in results, whereas slightly higher variability at lower concentrations (e.g., 1.56%) might be attributed to external factors or limitations in detection sensitivity at minimal levels of whey protein. Several studies have investigated the antimicrobial effects of whey protein and its derivatives on *Escherichia coli* (*E. coli*), including assessments of the minimum inhibitory concentration (MIC) (Shao *et al.,* 2020) showed that complexes formed between whey protein and ε-polylysine (ε-PL) exhibit antimicrobial activity against *E. coli*. The MIC of ε-PL in these complexes ranged from 11.72 to 25.00 μg/mL.

Figure (1) declared the effect of adding whey protein in two concentrations (1.56% and 3.13%) in yogurt manufactured in the laboratory and the results were very satisfying because of the growth of *E.coli* was completely inhibited through 24 hours in concentration 3.13% while in concentration of 1.56% the count decreased gradually till disappeared at the fifth day.

The count of *E.coli* in yogurt decreased which may be attributed to the accumulation of acid caused by the production of lactic acid and other organic acids, such as acetic acid, formic acid, etc., by yogurt starter bacteria (Ghaleh Mosiyani *et al.,* 2017). The obtained results were lower than (Karimi *et al.,* 2021) who found that the *E. coli* count in the samples was significantly decreased because the whey showed good antibacterial activity in the samples and by increasing the levels of bioactive peptides from yogurt whey, *E. coli* count was decreased. In a study by Aneta *et al.* (2020) found that the addition of 1% or 2% whey protein to yoghurt may be a good solution that can be routinely applied in the dairy industry to offer consumers a new functional product. Moreover, it offers a use for WP. Even though yoghurt's health benefits have long been recognized, ongoing attempts are being made to enhance its nutritional content as well as its functional and sensory aspects. Various polysaccharides and milk proteins are added for this purpose in the form of whey protein concentrates (WPC), skim milk powder, or whey powder (Sodini *et al.,* 2005; Yildiz-Akgül, 2018; Yildiz and Ozcan, 2019). Yogurt's rheological qualities, shelf life, and production costs can all be greatly enhanced by adding whey protein Glibowski and Rybak, (2016). As declared in Figure (2) the findings demonstrate that WP has a positive impact on most sensory attributes, particularly at higher concentrations (3.13%). However, the slight decline in the color score indicates that additional considerations may be needed to optimize the visual appeal of the product. Overall, WP shows significant potential as a functional ingredient for enhancing

sensory quality and consumer acceptability. These results were similar to Abd-El Naby *et al.* (2023) who reported that 2-4% WP increased the bacteriological quality to have numerous health benefits and improved sensory properties of bio-yogurt treatments compared to the control samples. These meet the rules and specifications set forth internationally. Thus, producing high-quality bio-yogurt is feasible. However, 4.5% of WP demonstrated the highest yoghurt quality, according to Zengjia *et al.* (2024), with good texture, increased viscosity, reduced syneresis, as well as superior particle size distribution and microstructure. Additionally, according to Ranok *et al.* (2021), 5% (w/w) WP was the best for making yoghurt and produced a satisfactory end product. The dairy industry may find that adding whey proteins to yoghurt is a smart way to give consumers a new functional product with health-promoting qualities. Even after 28 days of storage, the sensory quality and overall quality of yoghurts made with WP can be considered satisfactory Aneta *et al.* (2020). Owing to their nutritional, physical, and economic benefits, whey protein powders have been used as a fashionable trend for increasing the protein content of foods Tunick, (2008).

Biochemical study:

In the infected group, liver function test revealed significant elevation of ALT and AST activity ($P<0.05$) compared to the control group. On the other hand, the value of ALT and AST became similar to value of control group after supplementation of whey protein (Table 2).

Kidney function test revealed elevated serum urea and creatinine concentration in the infected group in comparison to control group ($P>0.05$). Unfortunately, adding the whey protein had no significant effect on urea and creatinine levels (Table 2).

E.coli infected group revealed significant lower values of total protein (TP), albumin and globulin ($P<0.05$) than control group. On the other hand, adding the whey protein restored the serum parameters to level similar to control group (Table 3).

Histopathological study:

The liver, kidney, spleen and intestine of the *E.coli* infected group, all showed variable pathological alterations. In the liver, marked congestion of the central vein was observed, consistent with vascular changes [Fig. 3a]. The hepatic sinusoids were dilated, with evident areas of hemorrhage characterized by erythrocytes extravasation into the parenchyma [Fig. 3b]. Centrilobular necrosis accompanied by vascular congestion were noted [Fig. 3c]. Vacuolar degeneration of hepatocytes was evident characterized by vacuolated cytoplasm, pyknosis and infiltration of inflammatory cells, including neutrophils and macrophages within the sinusoids and periportal areas [Fig. 3d]. Conversely, whey protein improved the pathological features of the liver compared to the infected group. Restoration of hepatic architecture enhanced vascular integrity and reduced hepatocyte degeneration, and necrosis were observed [Fig. 3e,f].

Histopathological analysis of the kidneys in *E.coli* infected rats showed significant structural alterations, including hemorrhage in both the renal cortex and medulla [Fig. 4a]. Coagulative necrosis characterized by eosinophilic cytoplasm and loss of normal architecture was observed with apoptosis which was evident as condensed and hyperchromatic nuclei in affected cells [Fig. 4b]. Widespread leukocytic infiltration, predominantly neutrophils and macrophages, was present in the interstitial tissue [Fig. 4c]. Degenerative changes such as cytoplasmic vacuolation and pyknotic nuclei were noted in some renal tubules [Fig. 4d]. In contrast, the whey protein-treated group showed less severe histopathological changes compared to the infected group [Fig. 4e,f].

Histopathological examination of the spleen in infected rats revealed significant splenic damage. Severe depletion of lymphocytes was evident in the white pulp [Fig. 5a]. Hemorrhage was observed within the red pulp with necrobiosis in the center of the white pulp [Fig. 5b]. Necrobiosis in the arterial wall was also observed [Fig. 5c]. In addition , leukocytic infiltration predominantly neutrophils and macrophages [Fig. 5d]. Rats treated with whey protein, exhibited significant improvement in splenic histopathology, including reduced lymphocyte depletion, decreased hemorrhagic lesions and preservation of splenic architecture [Fig. 5e,f]. Histopathological study of intestine in *E. coli*-infected rats revealed severe mucosal damage, characterized by degeneration and extensive inflammatory cell infiltration, primarily neutrophils and lymphocytes in the lamina propria [Fig. 6a]. Goblet cell hyperplasia was evident as a response to mucosal injury [Fig. 6b]. Sloughing of the upper tips of some intestinal villi was noted in severe damage [Fig. 6c]. Necrosis in some regions of the mucosa with hyperplasia of the intestinal gland lining were observed [Fig. 6d]. In the treated group, histopathological changes showed improvement, with evidence of mucosal repair and enhanced epithelial integrity [Fig. 6e, f].

The effects of *Escherichia coli* on various organs contribute to organ dysfunction and metabolic alterations through both direct impacts and immune-mediated tissue damage Ramaiah and Jaeschke, (2007). Because endotoxins build up to levels that damage the liver and cause these enzymes to leak, an *E. Coli* infection raised the levels of ALT and AST Salawu *et al.* (2018). The direct lethal effects of bacterial toxins and the influence of inflammatory mediators were demonstrated by this observation, which is in line with liver histological studies that show hepatocellular deterioration and necrosis Dinarello, (2000). Vascular dysregulation brought on by elevated cytokines and endotoxemia was suggested by the dilation of the hepatic sinusoids Fink and Warren, (2014). Moreover, endothelial injury is reflected in haemorrhage inside the hepatic parenchyma (Chambers & DeLeo, 2009). The immunological function of the liver in sepsis is further highlighted by the aggregation of inflammatory cells Ramaiah and Jaeschke, (2007). Renal impairment is suggested by elevated serum levels of creatinine and urea during an *E. coli* infection. In actuality, the endotoxin resulted in severe renal damage, raised blood urea levels and caused urea retention and accumulation. Since creatinine builds up in the bloodstream due to poor renal filtration rise creatinine levels (King *et al.,* 2007; Salawu *et al.,* 2018). According to Salawu *et al.* (2018), these biochemical results are consistent with histological alterations that show renal tubular injury in infected animals, such as leukocytic infiltration, coagulative necrosis, and vacuolar degeneration of renal tubular cells. Lower levels of albumin, globulin, and total protein were linked to *E. coli* infection. This decrease could be the consequence of hepatic dysfunction and the endotoxin-induced suppression of inflammation on protein production Salawu *et al.* (2018). Histopathological alterations in the spleen brought on by an *E.coli* infection include leukocytic infiltration, necrobiosis, haemorrhage, and lymphocyte depletion. The findings are in line with those of Al-Zamely and Falh (2011), who linked oxidative stress and the endotoxin effect to splenic injury (Long *et al.,* 2022). The gut histopathology of E. coli-infected rats demonstrated mucosal injury, inflammatory cellular infiltrates, goblet cell hyperplasia, degeneration and epithelial sloughing. Al-Zamely and Falh (2011) found similar findings and attributed this to the bacterial attachment to intestinal cells, which changed the shape of the cells. During bacterial infections, whey protein supplements protect several organs. The histopathological results, which show less organ damage, retained tissue integrity, and improved tissue

regeneration, are constant with the biochemical changes, such as decreased liver enzymes and restored protein levels. As the hepatocellular architecture improved, the whey protein-treated group's ALT and AST values dropped. Whey protein's bioactive components, including lacto-ferrin, immunoglobulins, and glutathione precursors, have anti-inflammatory and antioxidant qualities that protect the liver from damage brought on by oxidative stress Conceição *et al.* (2022).

Whey protein supports protein synthesis through its rich amino acid composition, restoring total protein, albumin, and globulin levels in rats. Furthermore, it improved immunological and metabolic balance by lowering inflammation and reversing the protein-depleting effects of *E. coli* infection Ebaid *et al.* (2015). Whey protein operates through numerous methods to protect the organs during bacterial infections. By raising the level of glutathione (GSH), it has an antioxidant effect. Additionally, it reduces the expression of cyclooxygenase-2 (COX-2) and interleukin-1β (IL-1β), which has an anti-inflammatory impact. By lowering oxidative stress indicators like nitric oxide (NOx), regulating immunological function, and encouraging tissue repair, tissue-regenerative qualities have been documented Xu *et al.* (2011) and Rehman *et al.* (2012).

The findings demonstrated that, in these experimental circumstances, whey protein had no influence on renal function or creatinine levels. The review by Levey and Coresh (2012) found that kidney disease progression varies widely among individuals and is influenced by some factors such as the severity of kidney damage and individual physiological responses. It highlights those improvements in tissue health (e.g., reduced inflammation or fibrosis) do not necessarily translate to improved glomerular filtration rate or overall kidney function. However, in rats with renal impairment caused by cyclophosphamide (CP), Mansour *et al.* (2017) found that whey protein recovered creatinine and urea levels in a dose-dependent manner. Additionally, it improved histological alterations such glomerular degeneration and tubular necrosis. Furthermore, a high dose resulted in full renal recovery. By blocking the programmed cell death pathway and re-establishing normal iron deposition in splenic macrophages, whey protein improves lymphocyte populations in the white pulp and exhibits immunomodulatory effects on spleen histology. Because it raises glutathione levels, it has antioxidant qualities and improves splenocyte protection and immunological response Ebaid *et al.* (2015). Lactoferrin and immunoglobulins are two of its bioactive components, which reduce damage and excessive inflammation during bacterial infections. According to Ebaid *et al.* (2015), these results demonstrate the potential use of whey protein as a natural antibacterial agent with immunomodulatory qualities for preserving gut integrity and health. In rats, *Escherichia coli (E. coli)* can cause oxidative stress and inflammation, which can result in major biochemical changes that are verified by histological abnormalities in different organs.

CONCLUSION

The current investigation shows that whey protein supplementation has strong inhibitory effects even at low dosages (1.56% and 3.13%). Additionally, it lessens harmful biochemical and histological alterations, which lessens the severity of *E. coli* infection in rats. The possible use of whey protein in food safety and health is the main topic of these findings. The yogurt supplemented with WP showed more palatability to consumers. We recommend that the precise bioactive ingredients in whey protein that cause these effects should be determined by more research, along with an

assessment of the protein's effectiveness against a wider variety of bacterial infections.

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استخدام بروتين مصل اللبن كمضاد بكتيرٌي في الزبادي ودوره في فئران التجارب المصابة بالا يشريكية القولونية عن طريق فحص التغيرات الباثولوجية وبعض مؤشرات الدم

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لقد اصبح هناك حاجة متزايدة لمواد حافظة مضادة للميكروبات جديدة في صناعة الأغذية، لذا بحث العمل الحالي في تأثيرات بروتين مصل اللبن على الإشريكية القولونية الملقحة في الزبادي المصنع في المختبر وأشارت النتائج التي تم الحصول عليها إلى أن إضافة بروتين مصل اللبن أدى إلى تثبيط كامل للإشريكية القولونية في اليوم الثالث بتركيز 3.13٪ بينما اختفى الميكروب في تركيز 1.56٪ في اليوم الخامس. كما تم تقييم الخصائص الحسية للزبادي أثناء التخزين في درجة حرارة الثلاجة (4 درجات مئوية) وكانت القبول العام للزبادي المدعم ببروتين مصل اللبن أكثر إرضاءً من الزبادي العادي. وعلاوة على ذلك، كان الهدف من هذه الدراسة هو التحقق في تأثير العدوى التجريبية بالإشريكية القولونية والمعالجة ببروتين مصل اللبن على مختلف المعايير الكيميائية الحيوية والتغيرات النسيجية المرضية في الفئران. لتحقيق هذا الهدف، تم تقسيم 18 فأرا ذكرًا إلى ثلاث مجموعات: 1) المجموعة الضابطة، 2) المجموعة المصابة (التي تم إعطاؤها 2 مل من محلول ملحي معقم بالفوسفات يحتوي على 1×10^9 وحدة مكونة لمستعمرات من الإشريكية القولونية لكل مل في اليومين السابع والرابع عشر)، و 3) المجموعة المعالجة (التي تلقت نفس البروتوكول مثل الحيوانات المصابة بالإشريكية القولونية، مع إضافة بروتين مصل اللبن بجرعة 2 مل / كجم يوميًا من اليوم 7 إلى اليوم 21 وحتى نهاية التجربة). في نهاية التجربة، تم جمع عينات الدم في ظل ظروف معقمة في أنابيب الطرد المركزي النظيفة للتحليل الكيميائي الحيوي و تم جمع عينات من الكبد والكلى والطحال والأمعاء للفحص النسيجي المرضي. وقد اثبتت التجربة ان اعطاء مصل اللبن بتركيز 1.56 و3.13% لها تاثير مثبط ويمكن أن تقلل من شدة الإصابة بالإشريكية القولونية والتغيرات النسيجية المرضية في الأعضاء المختلفة وقد يرتبط هذا الانخفاض بتعزيز إصلاح الأنسجة وتعديل الاستجابة المناعية وتقليل الالتهابات وتلف الخلايا.