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Functional and Nutritious Characteristics of Nanocurcumin-Enhanced Stirred Yogurt in Alloxan-Induced Diabetic Rats

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Abstract

The aim of this research was to evaluate the possible effect of stirred yogurt fortified with different levels of nanocurcumin (0.1 and 0.2%) on alloxan-induced diabetic rats.

The study involved 56 male Albino rats, randomly divided into seven experimental groups each group (n8). Both control negative group (1) and alloxan-induced diabetic positive control group (2) received a standard diet, while the alloxan-induced diabetic experimental groups group (3,4,5,6,7) received a standard diet plus (5 ml yogurt/day, 5mg Nano Curcumin (NC)/kg body weight, 10 mg (NC)/kg body weight, 5 ml yogurt fortified with 0.1 NC/day and yogurt fortified with 0.2 NC/day orally) respectively for 5 weeks.

Our results showed that fortification by different levels of NC (0.1 and 0.2%) did not significantly affect ($p \leq 0.05$) in the protein and fats contents as compared to the control stirred yogurt, the total solids were comparable in the tested samples and there are higher in the values of ash and acidity of the resultant stirred yogurt. Advancing storage period increased acidity and decreased pH. The lactic acid bacteria count in stirred yogurts increased in the presence of NC. The sensory evaluation revealed a significant effect ($p \leq 0.05$) by the applied treatments. Increasing levels of NC composite negatively influenced the sensory scores of the stirred yogurts, biological evaluation showed significantly observed ($p \leq 0.05$) in the serum levels of biochemical parameters and they significantly reduced blood glucose levels and lipid profile .The histopathological investigation of pancreas, liver and kidney in rats injected with alloxan and fed on stirred yoghourt fortified by different levels of NC had been improved than control (ve+) group. Our study found that administration of NC and stirred yogurt fortified by different levels of NC demonstrated anti-diabetic, dyslipidemia, anti-inflammatory effects on alloxan-induced diabetic rats.

Keywords: Keywords: Stirred yogurt; Nano Curcumin; Antioxidant; Hyperglycemia; Pancreas; Alloxan

1. Introduction

Diabetes is a metabolic illness that affects the body over time and is characterized by endocrine abnormalities and persistent hyperglycemia. **[1]; [2].** that occurs when the level of glucose in a person's blood rises because his body cannot create enough insulin hormone or cannot use the insulin it generates adequately **[3].** The persistent and varied signs of hyperglycemia include improper carbohydrate, fat, and protein metabolism [**4].** Hyperglycemia primarily contributes to the beginning and progression of these issues by the formation of reactive oxygen species (ROS), which causes lipid peroxidation and membrane damage. Obesity, hypertension, and dyslipidemia are frequent in diabetes patients, raising their risks for cardiac problems **[5].** About 80% of people with diabetes have type 2 diabetes (T2DM), the most prevalent kind of the disease. Insulin resistance is one of its characteristics **[6].** One representative material for type 1 diabetes is the β-cell toxin, or alloxan. ROS were produced by alloxan. Alloxan's beta cell-toxic effects arise from the redox process that produces free radicals **[7].** Diabetes and abnormal lipid profiles are significantly correlated, and this could lead to an increased risk of cardiovascular illnesses **[8].** Numerous studies using both humans and animals as models are examining how nutrition affects the treatment of type 2 diabetes **[9].** Yogurt is one of the functional foods that the public has recently been more interested in. Consuming yogurt has been linked to a lower risk of type 2 diabetes. This is because dairy products include significant levels

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of whey protein, calcium, magnesium, vitamin D, and certain fatty acids. Whey protein has been shown in certain trials to have glucose-lowering and insulinotropic effects **[10]**. Probiotics are another element in yogurt that has an antidiabetic effect in addition to these other substances. investigation by **[11]** which demonstrates that the probiotic bacteria *Lactobacillus lactis* have the potential to be used as antidiabetic probiotics due to their capacity to block the enzyme -glucosidase, which can lower the intestinal absorption of carbohydrates. Previous studies have demonstrated that adding natural components (herbs and spices) to yogurt can increase its effectiveness, provide value, and broaden its product line **[12].**

Customers frequently favour food goods that can enhance their quality of life as they become more conscious of the significance of diet for optimal health. As a result, functional foods have emerged and the market for their consumption is growing **[13].** Functional foods offer more than just nutritional benefits; they may also lower the chance of developing conditions like dyslipidaemia, cancer, type 2 diabetes, and cardiovascular disease (CVD) **[14]**. Curcumin is one useful substance that has preventative health effects against cardiovascular disease (CVD), the leading cause of death worldwide **[15].** Its exceptional antioxidant capacity slows the advancement of cardiovascular disease **[16].** Its prophylactic impact has been proven both in vivo and in vitro, and clinical tests have even verified it **[17].** *Curcuma longa* L. is an herbaceous plant in the *Zingiberaceae* family that has been used as a spice since 1900 BC. Curcumin is a yellow polyphenolic substance that is derived from the rhizome of this plant. It has also been utilized for ages as a traditional medicine to cure a variety of illnesses **[18].** Turmeric's primary bioactive ingredient, curcumin, is what gives the plant its strong anti-inflammatory, antibacterial, anti-mutagenic, and anticancer effects **Patel *et al*., (2020) [19].** The United States Food and Drug Administration (FDA) has declared curcumin safe for use as a spice, colouring, and flavouring agent **[18]**. In addition to rice, pasta, meat, vegetable, and salad dishes, it is frequently used in curries, mustard, sauces, drinks, cheeses, butters, and French fries **[20].**

The antidiabetic effects of curcumin and its potential to shield patients from macro and microvascular problems were covered in a review that was published by **[21].** Apart from potentially enhancing beta-cell function and decreasing inflammation, curcumin has also been shown to reduce the number of prediabetics who develop type 2 diabetes **[22], [23].** These days, there is a growing interest in examining the relationship between curcumin and glycaemic control indicators such insulin resistance **[24]**. For example, in overweight and obese T2DM patients, treatment with curcuminoids can considerably reduce fasting blood glucose and HOMA-IR **[25].** Nevertheless, curcumin's limited bioavailability poses significant challenges to reaping its benefits, mostly because of its poor water solubility, chemical instability in physiological circumstances, fast intestinal metabolism, malabsorption, and quick excretion from the body **[26].** And declared that "nanotechnology" will be the answer to reducing these difficulties. It appears that the use of nanoparticles may somewhat enhance curcumin's therapeutic role **[27],** However, delivery of nano-curcumin (NC) has greater promise for biological and pharmacological benefits **[28].** According to the description, the purpose of this study was to find out how nano curcumin affected the physicochemical, microbiological, and sensory aspects of stirred yogurt. Its impact on diabetic rats produced by alloxan as well as several other diabetes problems.

2. MATERIALS AND METHODS

2.1 Materials

For the dairy goods produced at the El Amer facility, fresh cow's milk was procured from milk suppliers. The local store provided the skim milk powder (96% T. S., 1.5% milk fat, 34% protein). Starter culture sold commercially culture for yogurt starters *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* were acquired from Danisco, Denmark, and are present in YO-MIX^{TM} 495 LYO 100 DCU. The analytical kits were procured from Bio-diagnostic Co. located in Dokki, Egypt. Memphis Company for Pharmaceutical and Chemical Industries, Cairo, Egypt, provided alloxan.

2.2 Experimental procedures

2.2.1 Preparation of Nano Curcumin

Nano Curcumin was obtained from Nano Fab Technology company, Giza, Egypt.

2.2.2 Production of stirred yogurt

Different types of stirred yogurt were produced in accordance with the protocol by **Tamime *et al*.** [**29].** with the following adjustments: Yogurt was made from cow's milk (fat 3.1%, protein 3.2%, total solids 12.3%, acidity 0.16%), to which 2% skim milk powder was added to boost the milk's solids. After three minutes of heating to 80°C, the mixture was quickly cooled to 45°C. Ten DCU per litter of milk was the addition rate for the functioning yogurt culture. After shaking and pouring the yogurt mixtures into 250-gram plastic cups, they were heated to 43 °C. The incubation period was extended to pH 4.7. At this stage, the yogurt was kept overnight in a refrigerator at 5±1ºC. The resulting stirred yogurt samples were kept for 14 days at 5±1ºC in the refrigerator. At intervals of 0, 3, 7, 10, and 14 days, the samples were examined.

2.2.3 Analytical methods

2.2.3.1 Chemical analysis of stirred yogurt:

The methods of **AOAC [30]** were used to determine the moisture %, protein %, fat %, and ash %. Each measurement was done in triplicate. The pH value of the stirred yogurt samples was determined electrometrically using a glass electrode lab pH meter (ADWA Model: AD1030; manufactured in Romania). The titratable acidity of stirred yogurt was calculated as lactic acid (TA%) according to Ling **[31].**

2.2.3.2 Microbiological examination of stirred yogurt:

MRS agar medium was used to count lactic acid bacteria, as stated in De Man et al. **[32]**., the coliform count was measured using Violet Red Bile Agar preparation and the plates were incubated at 37ºC for 48 hours according to APHA **[33]**, yeasts and moulds were counted on Malt-Extract Agar medium, the plates were incubated at 25–27°C For 4 days as suggested by Harrigan and McCance **[34]** .

2.2.4 Animal, housing and diets

An approximate weight of 180±5g, fifty-six male Albino rats were procured from the Agricultural Research Centre located in Giza, Egypt. The Laboratory Animal Unit, Faculty of Veterinary Medicine, Cairo University, Egypt, is where all the rats were housed. For eight weeks, the animal groups were maintained in an environment of filtered, pathogen-free air, water, and a temperature of 20–25oC with a 12-hour light/dark cycle, an 8–20-hour light cycle, and a 50% relative humidity. Every rat was fed a baseline diet for a week. 10% sucrose, 4% corn oil, 5% cellulose fibre, 3.5 percent mineral mixture, 1% vitamin mixture, 0.25 percent choline chloride, 0.3 percent D-L methionine, and 61.95 percent corn starch were the components of the basal diet **[35].** The Veterinary Institutional Animal Care and Use Committee (VET-IACUC), Faculty of Veterinary Medicine, Cairo University, Egypt, provided rules that were followed for all experimental operations. Throughout the course of the experiment, the rats were weighed once a week. following seven days of acclimatization before the research. Rats were divided into two major groups at random and then weighted. Eight rats in Group (1) control (-ve) were fed a regular meal exclusively; in contrast, 48 rats in Group 2 fasted all night and received a single intraperitoneal injection of freshly made alloxan at a concentration of 150 mg alloxan/kg body weight (BW) using citrate buffer 0.1M (pH = 4.5) as a vehicle **[36].** Diabetes was identified four days following the alloxan injection by utilizing a Glucometer (On Call Plus®) to detect blood glucose in tail vein blood. Rats deemed diabetic were those who's fasting glycemia was greater than 180 mg/dl, and they were chosen for the study **[37].** Rats were then split up into five groups. Group (2), the group with Alloxan-induced diabetes (+ve), was given merely a conventional diet. Group 3 was given 5 millilitres of yogurt every day via an epigastric tube. Group 4 received oral 5 mg N C/kg b.wt /day treatment **[38].** A solution of the necessary dosage was made in phosphate buffer (pH 7.4). Group 5 received oral treatment (10 mg N C/kg b.wt/day) **[39].** A solution of the necessary dosage was made in phosphate buffer (pH 7.4). Group (6) was given 5 millilitres of yogurt fortified with 0.1% of NC daily via an epigastric tube. Group 7 received 5 millilitres per day of yogurt fortified with 0.2% of N C via an epigastric tube.

2.2.5 Biological Determination

In accordance with **Chapman *et al.*, [40],** the biological evaluation of the various diets under test was conducted by calculating food intake (FI), body weight gain percentage (BWG%), and organ weight / body weight percentage. (Final Weight-Initial weight) / (Initial weight) = BWG% X 100

(Organ weight / Final weight) X 100 is the organ weight/body weight percentage.

2.2.6 Biochemical analysis

Using tiny capillary glass tubes, blood samples were extracted from the orbital plexus venous, put in centrifuge tubes without an anticoagulant, and left to clot. Following centrifugation (3000 rpm for 15 min) to prepare the serum, bio diagnostic kits were used to examine the serum samples, Serum glucose levels were measured using the procedure outlined by **[41].** The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured calorimetrically at 505 nm using a spectrophotometer (model DU 4700) in accordance with the method of **[41].** At 550 nm, total protein was measured using the procedure outlined by **Gornal *et al.*, [42].** The procedure of **Belfield and Goldberg, [43]** was followed to determine the alkaline phosphatase (ALP) activity calorimetrically using a spectrophotometer (model DU 4700) at 510 nm. By **Barham and Trinder, [44]**, serum uric acid was measured. utilizing a spectrophotometer set to 510 nm (model DU 4700). Serum urea nitrogen was measured using the procedure outlined in **Batton and Crouch 45].** use an adjusted wavelength of 550 nm spectrophotometer (model DU 4700). Serum creatinine levels were assessed using **Tietz** work **[46],** utilizing a spectrophotometer set to 510 nm (model DU 4700). The serum cholesterol was measured using a spectrophotometer (model DU 4700) set to 578 nm, in accordance with the procedure reported by **Allain *et al.*, (1974) [47].** Serum triglycerides (TG) were measured using the procedure outlined in **Fossati and Principe** work **[48].** utilizing a spectrophotometer set to 500 nm (model DU 4700). HDL-c, or high-density lipoprotein cholesterol, was measured using the procedure outlined in **Burstein [49].** utilizing a spectrophotometer set at 520 nm (model DU 4700). LDL-c, or low-density lipoprotein cholesterol, was measured using the procedure outlined in **Friedwald *et al.*, (1972) [50].**

2.2.7 Histopathology Technique

Following dissection, the liver and kidney tissue samples were promptly preserved in 10% neutral formalin for a whole day. Afterward, the samples were removed, dehydrated using an alcohol concentration, washed in xylene, and embedded in paraffin wax. Haematoxylin and eosin stains were applied to tissues after they had been sectioned at a thickness of 3 microns **[51].** and examined under a light microscope to look for any changes in the histopathology.

2.2.8 Statistical Analysis

The computer program SPSS software, version "20" for Windows, statistically subjected the data acquired from the present study to analysis of variance (ANOVA) in accordance with **Snedecor and Cochran [52].** To identify a significant difference between means, the least significant difference (LSD) value was employed. The data was shown as Mean ± SD. When a value was P ≤0.05, it was deemed significant; otherwise, it was deemed non-significant.

3. Results and discussion

3.1 Physicochemical properties of stirred yogurt fortified with different concentration of nanocurcumin.

The shelf life and quality of food are significantly influenced by its physicochemical properties **[53]**. Table 1 shows the protein, fat, ash, and total solids contents of the stirred yogurt that was enhanced with nanocurcumin, and the control stirred yogurt. The current study found that adding curcumin (at 0.1 and 0.2%) to stirred yogurt did not significantly difference ($p \leq 0.05$) the protein and fat levels when compared to the control stirred yogurt; this could be because of the small amount added. These findings corroborated those of **Kha *et al.* [54],** who discovered that over the course of the yogurt's 12-day storage, the fat content of the produced and collected yogurt somewhat decreased. The cause of this outcome could be lipid hydrolysis, and These outcomes align with the findings published by **Sardiñas-Valdés *et al*. [55].** who discovered that there were no appreciable variations between the curcumin-enriched cheeses and the control cheese in terms of protein or fat. In comparison to the control swirled yogurt (0.70±0.01), there was a significant ($p \leq 0.05$) difference in the ash contents of the fortification stirred yogurt with curcumin (at 0.1 and 0.2%) (0.75±0.01 and 0.78±0.01), respectively. Curcumin appears to become stable and functional upon interacting with transition metals such as Fe^{3+}, Mn^{2+}, Ni $^{2+}$, Cu $^{2+}$, Zn^{2+}, Pb $^{2+}$, Cd $^{2+}$, Ru^{3+}, and Re^{3+} **Zebib *et al*., (2010) [56]**. Understanding the mineral content of yogurt throughout processing is crucial as it impacts the physiochemical characteristics of the product.

Table (1) shows that there was a significant difference ($p < 0.05$) in total solids between the tested samples and the control. The range of total solids was 15.7 to less than 15.9. Yogurt control (15.9%), yogurt fortified with 0.1% nanocurcumin (15.7%), and yogurt fortified with 0.2% nanocurcumin (15.8%). **Qureshi *et al.* [57]** showed that all the yogurt samples had a rise in total solid % over time. A decrease in moisture was the cause of the increase in total solid%. In the control sample, the total solid % ranged from 14.60% to 14.75%. **EL- Muhammad, *et al.* [58]** calculated that 17.1% is the maximum range of total solids in yogurt. Over the storage of 15 days at 4°C, the solid contents of every sample rose. This rise in mass is attributed to the yogurt's loss of moisture. On the other hand, our findings diverge from those of **EL-Bannan *et al.* [59]** who discovered that T.S., Fat, Ash, and Protein were, in that order, 13.35, 3.25, 0.74, and 4.51.

Table (1): Chemical composition of stirred yogurt fortified with different concentrate of nanocurcumin.

Treatments	Chemical composition			
	protein	Fats	Ash	Total solids
Control	3.2±0.00^{a}	3.4±0.06^{a}	0.70±0.01^{d}	15.9±0.10^{a}
T1	3.2±0.00^{a}	3.4±0.06^{a}	0.75±0.01^{c}	15.7±0.10^{a}
T2	3.2±0.00^{a}	3.4±0.06^{a}	0.78±0.01^{b}	15.8±0.10^{a}

Data are presented as means ± SDM (*n=3*). a, b, c, d: Means with different letters among treatments in the same column are significantly different ($P \leq 0.05$) Control = yogurt without any addition, T1= yogurt fortified with 0.1% nanocurcumin, T2= stirred yogurt fortified with 0.2% nanocurcumin

3.1.1 Titratable acidity in yogurt

Table (2) display the titratable acidity variations that occurred in the stirred yogurt samples over the storage time. It was noticed that the stirred yogurts fortified with nanocurcumin had less acidity than the control sample. In terms of titratable acidity, there were no discernible variations ($P < 0.05$) between the stirred yogurts that contained nanocurcumin at concentrations of 0.2% and 0.1. Throughout the storage time, there was a small but non-significant increase in the titratable acidity of both the stirred and control yogurts containing nanocurcumin ($P < 0.05$). Our findings are consistent with previous publications. **Bakırcı and Kavaz [60], Bonczar *et al.* [61]** further stated that yogurts produced more acidity due to extended storage. The pH values of yogurts changed throughout storage, The pH value of stirred and control yogurts containing nanocurcumin declined during storage, in line with changes in titratable acidity. Since yeast also consumes sugar and organic acids, the pH level may drop. Several authors reported similar outcomes. **[62, 63].**

Table (2): Titratable acidity % of stirred yogurt fortified with different concentrate of nanocurcumin during storage at 5±1°C for 14 days.

Treatments	Titratable acidity% during storage periods (days)				
	Fresh	03	07	10	14
Control	0.74 ± 0.06^{aB}	0.77 ± 0.06^{aB}	0.81 ± 0.06^{aA}	0.85 ± 0.06^{aA}	0.88 ± 0.06^{aA}
T1	0.73 ± 0.02^{aC}	0.75 ± 0.02^{aB}	0.76 ± 0.02^{bB}	0.77 ± 0.02^{bB}	0.79 ± 0.02^{aA}
T2	0.73 ± 0.02^{aC}	0.75 ± 0.02^{aB}	0.76 ± 0.02^{bB}	0.77 ± 0.02^{bB}	0.79 ± 0.02^{aA}

Data are presented as means ± SDM (*n=3*). a, b, c, d: Means with different letters among treatments in the same column are significantly different ($P \leq 0.05$). A, B, C and D: Means with different letters among treatments in the same rows are significantly different ($P \leq 0.05$) Control = yogurt without any addition, T1= yogurt fortified with 0.1% nanocurcumin, T2= stirred yogurt fortified with 0.2% nanocurcumin.

The pH values of the stirred yogurts after 14 days of storage at 5±1°C are displayed in Table (3). Throughout storage, some variations in the pH levels of every sample were noted. pH values dropped while being stored. After 14 days of storage, the pH values decreased mostly as a function of temperature at 5±1ºC, particularly when stirring was done at pH 4.79. Regardless of the pH values after stirring at 4.79, regardless of storage temperature, the pH decreased significantly at 5±1°C. After stirring at pH 4.55, the pH, however, stayed at its lowest for 14 days. For example, after stirring the yogurt fresh at pH 4.79, 4.81, and 4.85 for control yogurt and fortified yogurt with nanocurcumin 0.1% and 0.2%, respectively, on day 14, the pH values in the yogurt were around 4.55, 4.65, and 4.96. There was a minor variation in pH values at day 14 between treatments, but it was not statistically significant ($P < 0.05$). The ingredients, the amount of milk, and the activity of lactic acid bacteria all affect the yogurt's pH.

Table (3): pH of stirred yogurt fortified with different concentrate of nanocurcumin during storage at 5±1°C for 14 days.

Treatments	pH during storage periods (days)				
	Fresh	03	07	10	14
Control	4.79 ± 0.10^{bA}	4.72 ± 0.10^{bB}	4.62 ± 0.10^{bC}	4.58 ± 0.10^{bD}	4.55 ± 0.10^{bD}
T1	4.81 ± 0.06^{aA}	4.77 ± 0.06^{aB}	4.72 ± 0.06^{aC}	4.69 ± 0.06^{aD}	4.65 ± 0.06^{aD}
T2	4.85 ± 0.06^{aA}	4.79 ± 0.06^{aB}	4.75 ± 0.06^{aC}	4.71 ± 0.06^{aD}	4.69 ± 0.06^{aD}

Data are presented as means ± SDM (*n=3*). a, b, c, d: Means with different letters among treatments in the same column are significantly different ($P \leq 0.05$). A, B, C and D Means with different letters among treatments in the same rows are significantly different ($P \leq 0.05$) Control = yogurt without any addition, T1= yogurt fortified with 0.1% nanocurcumin, T2= stirred yogurt fortified with 0.2% nanocurcumin.

3.1.2 Microbiological analyses of stirred yogurt fortified with different concentration of nanocurcumin.

3.1.2.1 Lactic acid bacteria count.

There has long been a connection between the health benefits of regular yogurt eating and the product's high concentration of live lactic acid bacteria **[64],65].** Because of this, minimum lactic acid bacteria levels have been set in several countries for yogurts and/or fermented milk products during their shelf lives. The range of these numbers is $1X10^6$ to $5X10^8$ CFU/g **[64].** Nonetheless, throughout the 14-day storage period at 5±1°C, the viability of the lactic acid bacteria strains (*Streptophilus* and *Lb*. *delbrueckii* ssp. *bulgaricus*) in all yogurt treatments exceeded the suggested minimum limits (10^7 cfu/ml or g). **FAO/WHO [66]** stated that 10^7 cfu/g or ml of live bacteria must be present in any food that is offered with health claims resulting from the addition of lactic acid bacteria. Yogurts that were kept fresher than plain yogurt had higher numbers of lactic acid bacteria (56 x 10^9cfu/ml) due to the presence of nanocurcumin. Between the 7and 14 days of storage, the viable lactic acid bacteria count rose in every sample of stirred yogurt. On day 14 of storage, the amount of viable lactic acid bacteria in stirred yogurt fortified with 0.1% and 0.2% of nanocurcumin (78 and 75 x 10^9 cfu/ml) was less than that of plain yogurt (93 x 10^9 cfu/ml). The rise in TTA as shown in Table (3) and the significant decrease ($p<0.05$) in pH on day 7 of storage as shown in Table (4) were correlated with the increases in viable cell counts for both yogurt bacteria throughout the first seven days of storage. Due to nanocurcumin's inhibitory effect on lactic acid bacteria development, there was a constant decrease in viable

cell counts in stirred yogurt supplemented with 0.1% (78 x 10^9cfu/ml) and 0.2% (75 x 10^9cfu/ml) when compared to the control treatment. These outcomes resemble **Buniowska-Olejnik *et al*., (2023)** [**67].** Because NC (0.2%) has antibacterial qualities and can support the essential activity of probiotic microbes, it can be utilized in yogurt formulations without compromising its nutritional value. In yogurt samples containing NC (mass fraction −0.1% to 0.25%), microbiological analysis was performed on the 7th, 14th, 21st, and 28th day of storage. The results showed that these additives not only exhibit antibacterial activity but also promote the growth and essential activity of the probiotic culture *Bifidobacterium animalis* subsp. *lactis* BB-12. The quantity of probiotics for NC was at the level advised for the duration of storage for functional fermented milk beverages (7–9 log CFU/g).

Table (4): Lactic acid bacteria count (10^9 CFU /ml) of stirred yogurt fortified with different concentrate of nanocurcumin during storage at 5 ± 1 ºC for 14 days.

Treatment	Lactic acid bacteria count (10^9 CFU /ml) during storage periods (days)				
	Fresh	03	07	10	14
Control	55 ± 2.64^{aE}	67.6 ± 0.57^{aD}	85 ± 4.35^{aC}	87.6 ± 2.0^{aB}	92.6 ± 4.0^{aA}
T1	43.6 ± 3.5^{bE}	51.3 ± 3.2^{bD}	64.3 ± 1.1^{bC}	76.3 ± 4.0^{cB}	77.6 ± 2.0^{bA}
T2	36.0 ± 2.0^{c}	42.6 ± 0.5^{cC}	58.3 ± 1.5^{cB}	74.6 ± 1.1^{bA}	75.0 ± 2.0^{cA}

Data are presented as means ± SDM (*n=3*). a, b, c and d: Means with different letters among treatments in the same column are significantly different ($P \leq 0.05$). A, B, C and D: Means with different letters among treatments in the same rows are significantly different ($P \leq 0.05$). Control = yogurt without any addition, T1= yogurt fortified with 0.1% nanocurcumin, T2= stirred yogurt fortified with 0.2% nanocurcumin

Results in Table (4) showed significant differences ($P \leq 0.05$) between control sample and treatments in There was no discernible change between treatments T1 and T2 for the whole storage period, except for 10 and 14 days. These findings concur with **Turek *et al*. [68].** He noted that there were no variations in the number of bacteria in the yogurts under test. But the effect of the 14-day storage period on the quantity of *Lactobacillus delbrueckii* ssp. *bulgaricus* bacteria.

3.1.2.2 Coliform count

Table 5 shows that no coliform bacteria were found in the fresh control or treatment stirred yogurts that were fortified with varying amounts of nanocurcumin and kept in a refrigerator for a period of 14 days. This might be because the various yogurt milks were heat-treated effectively (to 85ºC for three minutes) and because yogurt was manufactured and stored in very hygienic circumstances. Furthermore, the impact of acidity in various yogurts has been mentioned, which is crucial in lowering the pace at which coliform bacteria develop. These outcomes concur with **Negahdari *et al*., (2020) [69],** who discovered that NC (60 mg/mL) inhibited the growth of S. aureus ATCC6538, E. coli ATCC25922, and *E. faecalis* ATCC29212. Previous research indicates that curcumin nanoparticles increased aqueous-phase solubility and dispersibility over regular curcumin resulted in greater antibacterial activity **Shailendiran *et al*., (2011) [70].**

Table (5): Microbial quality (coliform count (10^1 CFU /ml) of stirred yogurt fortified with different concentrate of nanocurcumin during storage at 5 ± 1 ºC for 14 days.

Treatment	Coliform count (10^1 CFU /ml) during storage periods (days)				
	Fresh	03	07	10	14
Control	ND	ND	ND	ND	ND
T1	ND	ND	ND	ND	ND
T2	ND	ND	ND	ND	ND

Data are presented as means ± SDM (n=3). Control = yogurt without any addition, T1= yogurt fortified with 0.1 % nanocurcumin, T2= stirred yogurt fortified with yogurt fortified with 0.2 % nanocurcumin. ND= Not detected

3.1.3 Molds and yeasts count

Table (6) makes it evident that the number of moulds and yeasts grew gradually over the course of storage. Except for the control sample (20) after ten days, moulds and yeasts count did not detect CFU/ml in any of the fresh or treated yogurt samples at three days or later. After 14 days of storage, the greatest values were found in the control, T1, and T2 samples (45, 33, and 28), respectively. One possible explanation for the large number of moulds and yeasts is contamination via air inclusion during manual packaging. Additionally, the use of nanocurcumin after pasteurization and during product packing may cause post-contamination in yogurt samples.

The excessive number of moulds and yeasts may be caused by air contamination that occurred when the various yogurt treatments were being stirred. The results are in harmony with the results of **Con *et al.* [71].** and **Zakai and Erdogan [72]** who stated that the yeast and mould count in all the yogurt samples had significantly increased. Yogurt sample counts for yeast and mould varied from 2.10 to 2.89 cfu/g. Yogurt with grape molasses and control had the lowest yeast and mould count at first, but after 10 days, the counts were nearly identical to those of the other yogurts. It is evident that the numbers of mould and yeast grew over the course of storage. The addition of fruit taste raised the numbers of mould and yeast.

Table (6): Microbial quality (Molds and yeasts count (10^2 CFU /ml) of stirred yogurt fortified with different concentrate of nanocurcumin during storage at 5 ± 1 ºC for 14 days.

Treatment	Molds and yeasts count (10^2 CFU /ml) during storage periods (days)				
	Fresh	03	07	10	14
Control	ND	ND	ND	20	45
T1	ND	ND	ND	12	33
T2	ND	ND	ND	9	28

Data are presented as means ± SDM (n=3). Control = yogurt without any addition, T1= yogurt fortified with 5 % nanocurcumin, T2= stirred yogurt fortified with 0.1% nanocurcumin, T2= stirred yogurt fortified with 0.2% nanocurcumin. ND= Not detected

3.1.4 Sensory evaluation of yogurt fortified with different concentrate of nanocurcumin.

A team of judges evaluated yogurts based on their sensory qualities and assigned scores based on several attributes to determine consumer approval. The scores were distributed as follows: 10 for flavour, 10 for body and texture, 10 for colour, and 10 for acceptance overall. Table (7) displays the yogurts' sensory attributes. Throughout storage, the expert panel expressed a preference for stirred yogurts with 0.1 and 0.2% nanocurcumin composite or control stirred yogurt. The sensory scores of the stirred yogurts were adversely affected by rising concentrations of nanocurcumin compound. When it came to look during storage, Stirred Yogurt (Control) was the favourite. During storage, no significant differences were found for flavour or overall acceptance of any of the stirred yogurt samples; however, for stirred yogurts (control), significant differences were found for colour, body, and texture. Stirred yogurt T1 had the lowest overall acceptance, colour, body, and texture (8.3±1.16b, 8.6±1.04b, and 8.6±0.84a), respectively. There were no discernible variations in the flavour, colour, body and texture, or acceptance of the stirred yogurt enhanced with nanocurcumin. These outcomes concur with **Joseph *et al.*, (2011) [73],** Researchers found that the overall acceptance of the yogurt product was significantly influenced ($p<0.05$) by the flavour in terms of taste and smell. Therefore, for better consumer acceptability, yogurt manufacturers need to enhance the sensory qualities, including flavour and taste. Additionally, by labelling according to specifications that accurately reflect the kind and content, they may enhance the packaging. **Zekai, (2010) [74],** stated that there were no significant differences between the treatments in terms of taste, fragrance, or perceived sweetness. Additionally, it was noted that the duration of storage had varying effects on every panellist across all sensory metrics assessed. Characterizing the texture of yogurt is crucial for quality assurance, process and product development, and consumer acceptability. Measurements obtained through instrumental or sensory means can be used for this characterisation.

Table (7): Sensory evaluation of stirred yogurts fortified with different concentrate of nanocurcumin

Character assessed	**Treatments**		
	Control	T1	T2
Flavor (10)	8.9±0.99[a]	8.4±1.07[b]	8.4±1.26[b]
Body & texture (10)	9.0±0.86[a]	8.6±1.04[b]	8.8±1.15[b]
Color (10)	9.6±0.52[a]	8.3±1.16[b]	8.5±1.51[b]
Overall Acceptance (10)	9.15±0.75[a]	8.6±0.84[b]	8.7±0.82[b]

Data are presented as means ± SDM (n=10). a, b, c, d: Means with different letters among treatments in the same rows are significantly different ($P \leq 0.05$) Control = yogurt without any addition, T1= yogurt fortified with 0.1 % nanocurcumin, T2= stirred yogurt fortified with 0.2 % nanocurcumin.

3-2 Biological Evaluation of nanocurcumin and stirred yogurts fortified with different concentrate of nanocurcumin.

3.2.1- Biochemical Analyses

Data illustrated in Fig (1) showed significant increase ($P \leq 0.05$) in the level of glucose (346.33±5.54) of control (ve+) group, which treated with a single dose of alloxan, compared to control (ve-) group (97.66±2.02). Similar results have been

obtained by **Radhika *et al.* [75]** demonstrated that alloxan administration was associated with hyperglycemia by creating a repeatable type of diabetes mellitus with low beta cell function. Alloxan administration resulted in a significant increase in glucose levels compared to the control and treated groups, and treatment with nanocurcumin resulted in a decrease in plasma glucose levels; this reduction in plasma glucose by curcumin is linked to an increase in glucokinase activity.

And may be this reduction relates to curcumin antioxidant activity, after five weeks of treated by NC and stirred yoghurt fortified with different concentration of NC showed reduce the level of glucose in (yoghurt fortified with 0.2% NC, yoghurt fortified with 0.1% NC, 10mg NC, 5mg NC and yoghurt only) groups, respectively (97.33±1.76, 106.00±2.08, 112.33±1.85, 131.66±2.02 and 223.00±2.08), respectively. Furthermore, the highest reduction in glucose level was in the animal groups affected by alloxan and fed with (yoghurt fortified with 0.2% NC) and (yoghurt fortified with 0.1% NC) groups. Curcumin reduces circulating free fatty acids (FFAs). FFA-induced lipo-toxicity is a significant contributor of insulin resistance. This mechanism has been proposed to deteriorate pancreatic β-cell function **[76, 77]** demonstrated that oral dose of curcumin and nanocurcumin reduced glucose levels while increasing insulin serum concentration. The difference between the curcumin and control diabetic groups was not significant. It means that nano-curcumin can reduce the negative effects of diabetes on glucose and insulin.

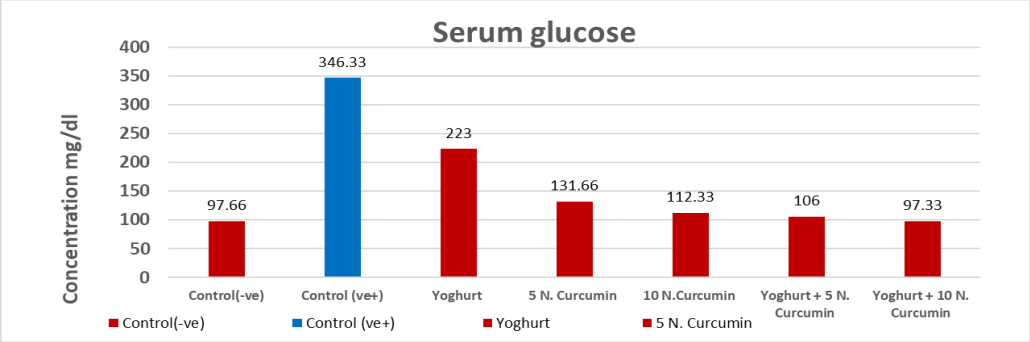

Fig. (1): Effect of NC and stirred yogurt fortified with different ratio of NC on serum blood glucose concentration (mg/dl) of alloxan-induced diabetic rats.

Table (8) shows the results of several liver enzymes for each group. At the end of the experiment, the serum levels of ALT, AST, and ALP in the diabetic rats induced by alloxan control (ve+) group were significantly (P≤0.05) higher than those of the control (ve-) group (39.23±1.17, 62.33±1.45, and 105.00±1.73, respectively). The levels of ALT, AST, and ALP were significantly (P≤0.05) lower in the oral intake of NC and stirred yogurt fortified with different ratios of NC than in the (yoghurt fortified with 0.2% NC, yogurt yoghurt fortified with 0.1% NC, 10mg NC, 5mg NC, and yogurt only) groups, respectively. In the end, it was evident from the same Table (8) that the groups with yoghurt fortified with 0.2% NC and yoghurt fortified with 0.1% NC had the greatest reduction. Fermentation may enhance the milk's nutritional content. For example, during the fermentation of milk, ***Streptococcus thermophilus*** can create folate and some bacteria can synthesize vitamins B **Pei *et al.*, (2017) [78]**.

Table (8): Liver enzymes (U/I) of alloxan-induced diabetic rats' groups

Parameters (U/I)	Groups						
				NC (mg / kg)		Yogurt +	
	Control (ve-)	Control (ve+)	Yogurt (g)	5	10	0.1% NC	0.2% NC
ALT	39.23±1.1e	159.33±2.3a	114.00±2.08b	64.33±1.45c	51.66±1.45d	51.66±0.67d	39.00±1.15e
AST	62.33±1.4e	190.66±2.9a	95.00±1.73b	81.33±2.33c	74.66±1.45d	71.00±0.58d	64.00±1.15e
ALP	105.00±1.ef	250.00±2.5a	167.00±2.00b	135.00±1.1c	116.33±1.3d	107.66±2.0e	103.33±1.33 f

Data are presented as means ± SDM (*n=8*). Data in the same row with different superscript letters are statistically different ($P \leq 0.05$). NC: Nanocurcumin AST: aspartate amino transferase ALT: alanine amino transferase ALP: alkaline phosphatase

The kidney function results for each group are shown in table (9). Serum urea, creatinine, and uric acid levels in diabetic rats induced by alloxan control (ve+) group increased significantly (P≤0.05) until the end of the experiment (61.66±1.82,

1.74±0.04, and 3.73±0.12, respectively) compared with the_control (ve-) group (33.33±1.85,0.67±0.01, and 2.20±0.15, respectively). The current study's results closely align with those of **Zevallos *et al.* [79],** which showed that renal damage in hypercholesterolemia may be associated with an increase in serum urea nitrogen levels, a sign of both glomerular and tubular kidney failure. Increased levels of urea and creatinine suggest renal impairment and/or protein catabolism. **Abdel-Wahhab *et al.* [80].** There was a substantial (P≤0.05) decrease in blood urea, creatinine, and uric acid in the oral NC consumption and stirred yogurt supplemented with varied concentrate of NC, as well as in the yoghurt fortified with 0.2% NC, yoghurt fortified with 0.1% NC, 10 mg NC, 5 mg NC, and yogurt only) groups. Ultimately, it was evident from the identical Table (9) that the groups that received yoghurt fortified with 0.2% NC and yoghurt fortified with 0.1% NC had the greatest reduction. Recent research has indicated that curcumin exhibits nephroprotective action as well as therapeutic potential against renal disorders. Furthermore, it has been documented that curcumin has nephroprotective benefits for renal impairment in several experimental scenarios, including those involving cisplatin, acetaminophen, nephrectomy, and gentamicin **[81]. Rebholz *et al.* [82**] found that participants with anti-inflammatory and antioxidant qualities that affect kidney health had a decreased risk of developing renal disease.

Table (9): Kidney function (mg/dl) of alloxan-induced diabetic rats' groups

Parameters (mg/dl)	**Groups**						
	Control (ve-)	Control (ve+)	Yogurt (g)	NC (mg / kg)		**Yogurt +**	
				5	10	0.1%NC	0.2% NC
Urea	33.33±1.85^{e}	61.66±1.82^{a}	56.66±1.20^{b}	49.50±0.2^{c}	43.66±1.4^{d}	43.66±2.0^{d}	32.00±1.1^{e}
Creatinine	0.67±0.01^{e}	1.74±0.04^{a}	1.00±0.05^{b}	0.88±0.05^{c}	0.89±0.06^{c}	0.75±0.07^{d}	0.73±0.06^{d}
Uric Acid	2.20±0.15^{d}	3.73±0.12^{a}	3.16±0.08^{b}	2.66±0.12^{c}	2.46±0.14^{c}	2.23±0.03^{d}	2.20±0.11^{d}

Data are presented as means ± SDM (*n=8*). Data in the same row with different superscript letters are statistically different (P ≤ 0.05). NC: Nanocurcumin

In the current studies, alloxan-induced diabetic rats exhibited a significant increase (P ≤0.05) in total triglycerides (TG), low-density lipoprotein (LDL-c), and total cholesterol (TC) when compared to the control (ve-) group. However, there was a significant decrease (P ≤0.05) in HDL-c. Table (10) shows that the lack of insulin in diabetic patients may be the cause of this rise in TG. The results obtained were consistent with those of **Hassan and Emam [83]** and **Isa *et al.* [84].** In comparison to normal control rats, the animals injured by alloxan had notable increases in their hepatic TG, TC, and LDL levels, but a decrease in their HDL value.

Table (10): Lipid Profile (mg/dL) of alloxan-induced diabetic rats' groups

Parameters (mg/dL)	**Groups**						
	Control (ve-)	Control (ve+)	Yogurt (g)	NC (mg / kg)		Yogurt +	
				5	10	0.1% NC	0.2%NC
TC	95.46±0.7^{e}	161.33 ±2.96^{a}	123.33±1.^{b}	113.00 ±0.58^{c}	101.00 ±1.15^{d}	101.33±1.4^{d}	96.66±1.20^{e}
TG	45.33±1.20^{f}	139.33 ±0.88^{a}	89.00±2.08^{b}	85.66 ±1.20^{c}	56.33 ±1.45^{d}	50.66±2.33^{e}	45.66±0.33^{f}
HDL-$_{c}$	65.30±1.1^{b}	26.33 ±0.88^{g}	40.33±0.88^{f}	46.66 ±1.85^{e}	49.00 ±0.57^{d}	56.00±2.51^{c}	68.6±12.4 ^{a}
LDL-$_{c}$	12.90±0.32^{f}	79.80 ±2.33^{a}	62.80±1.33^{b}	26.80 ±1.30^{c}	15.20 ±0.50^{e}	17.26±1.56^{d}	13.40±0.46^{f}

Data are presented as means ± SDM (n=8). Data in the same row with different superscript letters are statistically different (P ≤ 0.05) NC: Nanocurcumin, LDL-C: Low density lipoproteins cholesterol. HDL-C: Serum high density lipoproteins cholesterol; TC: Serum total cholesterol; TG: Serum triglyceride.

Significant (P≤0.05) reductions in hepatic concentrations of TG, TC, and LDL were observed after treatment with NC and stirred yogurt fortified with varying concentrate of NC, along with a rise in HDL levels. These outcomes are consistent with **Dadgar *et al.* [85].** They showed that as compared to the diabetic control group, the administration of curcumin in the form of nano improved the lipid profile of the diabetic rats, which is consistent with findings from earlier studies **[86, 87].**

3-3 Histopathological Examination:

3.3.1 Histopathological examination of pancreas:

Microscopically, the pancreas of rats from the control (ve-) group showed normal pancreatic acini and islets of Langerhans. Otherwise, the pancreas of rats in the control (ve+) group showed vacuolation of islets of Langerhans's cells (black arrow) and hyperplasia in the epithelial lining of the pancreatic duct (red arrow) (Fig. 2). Alloxan selectively causes pancreatic β-cell death by producing cytotoxic ROS **[88].** Alloxan causes necrosis of pancreatic beta cells and stimulates free radical generation, both of which contribute to the pathophysiology of experimental and human diabetes mellitus **Soto *et al*., (2004) [89].** Alloxan readily and rapidly accumulates in pancreatic beta cells **[90].** and accumulating alloxan causes aberrant changes in membrane potential and ion channels in pancreatic beta-cells **[91].**

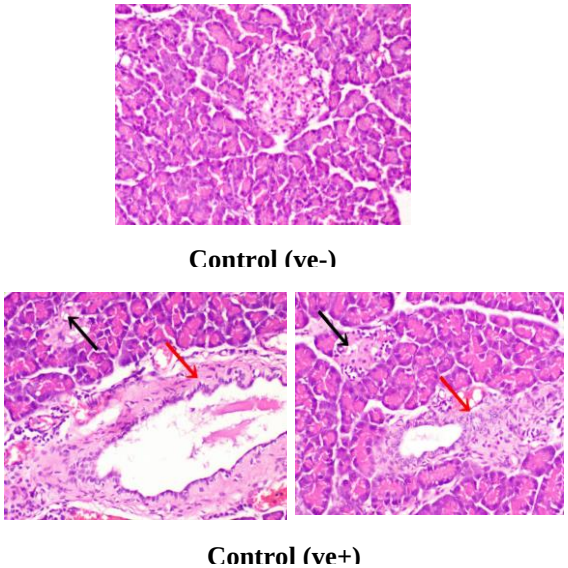

Fig. (2): Photomicrograph of Pancreas in control (-ve) and control (+ve) groups (H&E)

Meanwhile, the pancreas of rats in the yogurt group showed vacuolation of islet of Langerhans cells. Furthermore, slices from rats in the N. Curcumin (5mg) group showed vacuolation of certain islets of Langerhans cells (black arrow) and congestion of pancreatic blood vessels (red arrow). Furthermore, the pancreas of rats in the N. Curcumin (10mg) group revealed necrosis or vacuolation of several Langerhans islet cells. On the other hand, several analysed sections from the yoghurt fortified with 0.1% NC and yoghurt fortified with 0.2% NC groups showed no histological changes (Fig. 3). Curcumin is a polyphenolic substance that decreases glycemia and hyperlipidaemia, as well as its anti-inflammatory and antioxidant characteristics, which have favourable effects on diabetic complications **[92].**

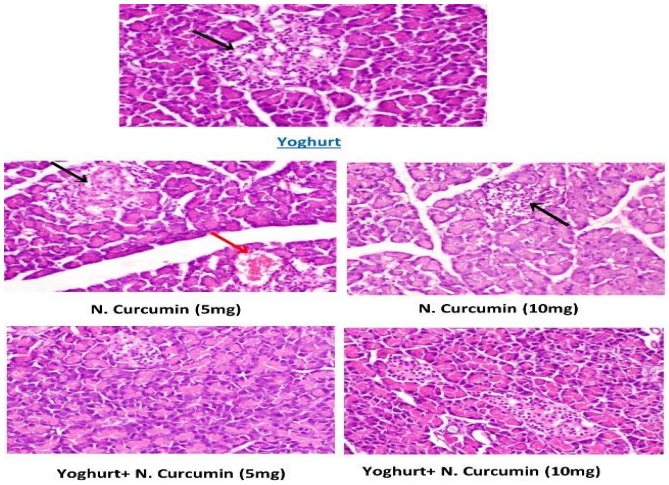

Fig. (3): photomicrographs of haematoxylin – eosin-stained Pancreas in all experimental rats

3.3.2 Histopathological examination of liver:

Microscopic analysis of liver sections from rats in the control (ve-) group revealed normal histological architecture of the hepatic lobules. In contrast, the liver of rats in the control (ve+) group showed proliferation of Kupffer cells (black arrow), focal hepatocellular necrosis associated with inflammatory cell infiltration (red arrow), and fibroplasia in the portal triad (blue arrow) (Fig. 4).

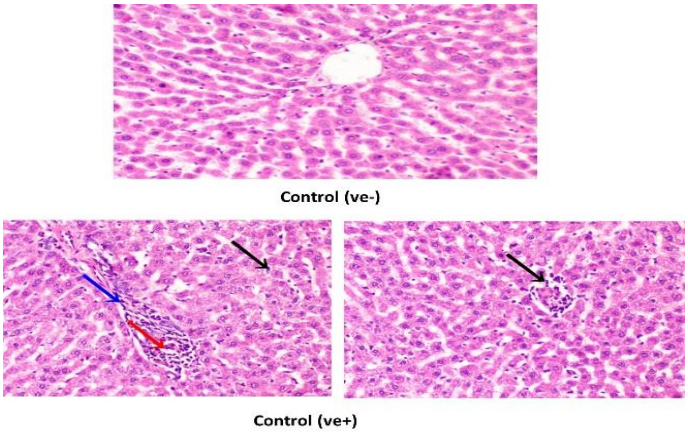

Fig. (4): Photomicrograph of liver in control (-ve) and control (+ve) groups (H&E)

Meanwhile, the livers of rats in the yogurt group showed minor proliferation of Kupffer cells (black arrow) and slight dilation of hepatic sinusoids (red arrow). In contrast, the liver of rats in the N. Curcumin (5mg) group showed mild fibroplasia in the portal triad (black arrow) and slight oval cell proliferation (red arrow). Otherwise, rats' livers from the N. Curcumin (10mg) and (yoghurt fortified with 0.1% NC)) groups revealed slight proliferation of Kupffer cells (black arrow). However, the yoghurt fortified with 0.2% NC group showed histologically normal hepatic tissue (Fig. 5). Curcumin is anti-inflammatory because it inhibits important inflammatory enzymes like cyclooxygenase-2, lipoxygenase, and inducible nitric oxide synthase **[93].** Curcumin is an antioxidant because it inhibits the generation of ROS and stimulates endogenous antioxidant activity. Curcumin's unusual, conjugated structure gives it the characteristic radical-trapping properties of a chain-breaking antioxidant **[94].** Recently, **El-Gizawy *et al*. [95]** Nanocurcumin has been shown to have hepatoprotective and therapeutic properties due to structural and functional effects on the liver. They demonstrated that nanocurcumin reduces hepatic cord disorganization, portal triad fusion, and central vein congestion while also decreasing liver function indicators. They attributed these effects to potent antioxidant and anti-inflammatory roles of nanocurcumin.

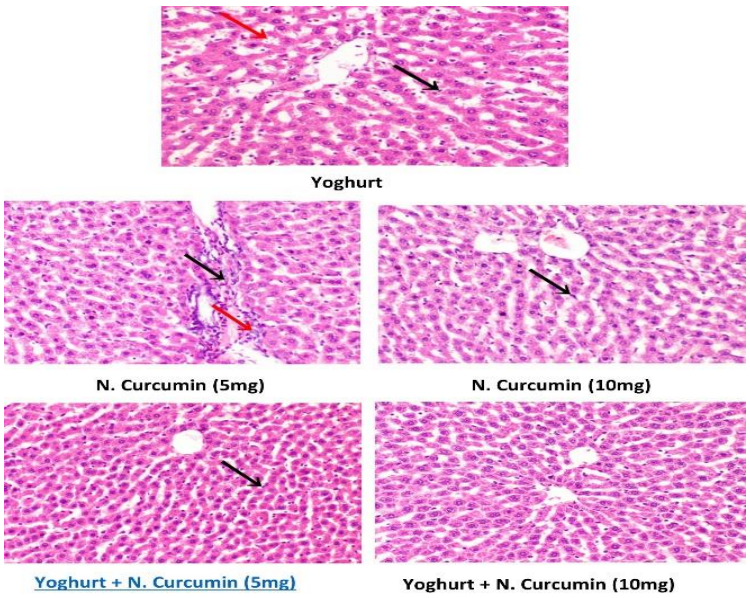

Fig. (5): photomicrographs of haematoxylin – eosin-stained Liver in all experimental rats

4. Conclusion

Based on the results, it could be concluded that consumption of nanocurcumin and stirred yoghourt fortified by different concentrate of nanocurcumin demonstrated anti-diabetic, dyslipidaemia, anti-inflammatory effects on alloxan induced experimental rats.

5. Funding:

Not applicable

6. Conflicts of interest

The authors declare that they have no conflict of interest

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