



Therapeutic effect of functional drinking yoghurt fortified with aqueous *Cassia fistula* pods extract on injured liver rats by cadmium chloride

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ABSTRACT

This study aimed to investigate the impact of fortifying drinking yoghurt with an aqueous extract of *Cassia fistula* pods (AECFP) at ratios 3, 6 and 9% on the chemical composition and sensory attributes of manufactured yoghurt. After that, the therapeutic effect of produced functional yoghurt on liver damage induced by cadmium chloride in albino rats was assessed. The results obtained revealed that AECFP had high antioxidant activity and total phenolic and flavonoid content. Polyphenol analysis identified ferulic acid and ellagic acid as the predominant compounds in *Cassia fistula* pod extract. In fortified drinking yoghurt, increasing AECFP ratios significantly affected moisture content, protein, fat, and ash levels, while color measurements showed decreased lightness and increased redness and yellowness with higher AECFP ratios. Sensory evaluation indicated improved color, flavor, and texture with higher AECFP levels, peaking at 9% AECFP. In hepatotoxic rats, drinking yoghurt, particularly with 9% improved body weight gain and food intake compared to the positive control. The yoghurt fortified with 9% AECFP also led to significant improvements in liver and kidney function, with reductions in liver enzymes and bilirubin levels, and lower urea and creatinine levels. Additionally, the fortified yoghurt positively influenced lipid profiles, reducing triglycerides, total cholesterol, LDL-c, and VLDL-c, while increasing HDL-c. Antioxidant enzyme levels showed improvement in hepatotoxic rats receiving fortified yoghurt, indicating enhanced oxidative stress mitigation. Histopathological examination revealed significant restoration of liver and kidney tissue architecture in rats receiving yoghurt fortified with 3% and 9% AECFP. These findings demonstrate that drinking yoghurt fortified with *Cassia fistula* pod extract can enhance the sensory qualities of the yoghurt while providing notable health benefits, particularly in mitigating oxidative stress and organ damage in hepatotoxic conditions.

Keywords: *Cassia fistula*, drinking yoghurt, sensory evaluation, hepatotoxic rats, cadmium chloride, histopathology

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INTRODUCTION

Cassia fistula is a beautiful ornamental tree because of its yellow and bright flowers. It belongs to the Fabaceae family (Saxena *et al.*, 2011), and Golden Shower, Pudding Pipe and India Laburnum tree are some of the common names for this tree. *Cassia fistula* is widely grown in tropical and subtropical climates, particularly in India and Sri Lanka, and also diffuses in more countries such as Mexico, China, Egypt, Thailand, Brazil, East Africa, and South Africa

(Bahorun *et al.*, 2005, Danish *et al.*, 2011 and Mishra *et al.*, 2024). The *Cassia fistula* seeds extract with high LC50 value signified that this plant is not toxic to human (Lachumy *et al.*, 2010).

Since prolonged times, all parts of the *Cassia fistula* tree have been used as herbs in folk remedies and for many medicinal purposes as antipyretic, anti-inflammatory, antitumor, antioxidant, analgesic and hypoglycemic, also effective against tuberculosis (Kulkarni *et al.*, 2015, Pawar *et al.*, 2017 and Sanoria *et al.*, 2020). The root is used to treat fever, constipation, biliousness, heart diseases, joint pain, and chest pain. The leaves, buds and extracts are used for treatment of skin diseases, rheumatism, eczema, ringworms, tussive and burns. While flowers are used as a diuretic. *Cassia fistula* pods are used as a laxative and a diabetes treatment. Further, its pulp is used to treat cardiac conditions, stomach problems, urinary disorders, abdominal and hepatoprotective. The seeds are known to be an appetizer, laxative and carminative. They are also used as an effective treatment for jaundice, oral sores, swollen throats and biliousness. So, *Cassia fistula* is known as a killer of disease (Kumar *et al.*, 2017, Maqsood *et al.*, 2020 and Mwangi *et al.*, 2021).

Cassia fistula fruit is rich in bioactive compounds such as polyphenolic, flavonoid compounds, anthraquinones, glycosides, carbohydrates, kaempferol, anthraquinones, 4-hydroxy benzoic acids hydrate, volatile components, ascorbic acid, proteins, amino acids and essential oils (linoleic, oleic and stearic) (Danish *et al.*, 2011, Irshad *et al.*, 2012 and Kumar *et al.*, 2017). The leaves mainly contain oxalic acids, tannins and anthraquinones derivatives (Kushawaha and Agrawal, 2012). Hence, *Cassia fistula* parts or its extracts can be used in the production of some functional foods to reduce or prevent hepatotoxicity risk because of their content of bioactive compounds and their health benefits.

The liver is the responsible organ for the detoxification of the body and it has an essential role in the metabolism process. Any liver damage will affect its function and may lead to long-term liver injury hence liver fibrosis and carcinoma. The common liver illnesses in the world, especially in developing countries are hepatitis (A, B, C and E), fatty liver, cirrhosis and cancer (Sivakrishnan and Pharm, 2019). Hepatotoxicity is meaning any damage to the liver or liver dysfunction as a result of intake or exposure to immoderate doses from some chemicals and xenobiotics such as drugs (Singh *et al.*, 2016), pesticides, food additives, alcohol, heavy metals, chlorinated solvents, peroxides, fungal toxins and radioactive isotopes (Singh *et al.*, 2011). Long-term accumulation of chemicals and foreign substances leads to a variety of symptoms ranging from asymptomatic hepatitis, acute hepatitis, chronic hepatitis and liver failure (Cano *et al.*, 2017 and Gulati *et al.*, 2018). Several studies declared that functional food consumption led to lower inflammatory and oxidative hepatic damage and decreased liver triglycerides (Bakhshimoghaddam *et al.*, 2018, Mohamed *et al.*, 2018 and Ali, 2022).

Fermented dairy products, particularly yoghurt, which is made by lactic acid bacteria (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*) fermenting milk at 37°C, are among the most significant functional foods (Hadjimbei *et al.*, 2022). According to their physical and textural characteristics, the most popular yoghurt products in markets are divided into three categories: set, stirred, and drinkable (Gunawardhana & Dilrukshi, 2016 and Sobhay *et al.*, 2019). All yoghurt types are very nutritious and have useful healthy effects on metabolic processes to promote human health and prevent diseases by controlling body weight, energy balance and blood sugar control (Mahmoud *et al.*, 2021).

A few studies showed that yoghurt fortification with some foods such as fruits, vegetables, herbs and medicine plants or its extracts rich with bioactive compounds led to an increase in its healthy effects and therefore reduced or prevented the development of hepatic disorders (**Abd El-Ghany *et al.*, 2012** and **Al-Soudy *et al.*, 2020**). More researches are needed to clarify and confirm that. Thence, this paper aimed to prepare drinking yoghurt fortified with the aqueous extract of *Cassia fistula* pods (AECFP) and how fortified yoghurt might lessen liver-related damages in rats induced with cadmium chloride (CdCl₂).

MATERIALS AND METHODS

Ethical statement

This study was carried out with permission from Zagazig University's institutional animal care and research unit (Institutional Review Board Number ZU-IACUC/2/F/293/2023).

Materials and ingredients

Fresh *Cassia fistula* pods were procured from a local market and identified by a botanist for authenticity. Buffalo skim milk was procured from a dairy supplier for the Dairy Technology Unit, Faculty of Agriculture, Zagazig University. Lactic acid bacteria cultures used in this study, including *Lactobacillus delbrueckii* subsp. *bulgaricus* (EMCC1102) and *Streptococcus salivarius* subsp. *thermophiles* (EMCC1044), were obtained from the Laboratory of Microbiology, Faculty of Agriculture, Ain Shams University, Egypt. Thirty adult albino Wistar rats (weight 120±10 g) were purchased from Helwan Breeding Farm, Cairo, Egypt. The basal diet ingredients (casein, starch, cellulose, vitamins, minerals and choline chloride) and a few kits for biochemical analysis were procured from El-Gomhoria Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt. From Sigma (St. Louis, Missouri, USA), cadmium chloride (CdCl₂), gallic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were brought.

Preparations of aqueous extract of *Cassia fistula* pods (AECFP)

Cassia fistula pods were washed by tap water then desiccated, and ground into a fine powder. The powdered pods were blended with distilled water at a 1:3 w/v ratio, left overnight at room temperature, and then filtered using cheesecloth to separate the rough parts. The filtrate was divided into three parts; the first part was saved in the freezer at -20 °C until chemical analysis. The second part of the extract was evaporated under reduced pressure, using a rotary evaporator (BÜCHI-water Bath-B-480, German) to concentrate it, then freeze-dried by a Freeze Dryer (France type) at -58.2°C, then kept in a freezer at -20 °C till polyphenol analysis. The third part was pasteurized at 72 for 15 sec and added to the drinking yoghurt.

Drinking yoghurt preparation

All drinking yoghurt treatments were produced using **Thomas and Wansapala (2017)** methodology with a few adjustments. In the first step, the yoghurt was manufactured as follows: Buffalo skim milk was heated at 85°C for 10 min, then cooled to 42± 2°C, before adding 3% yoghurt culture including *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *Thermophilus*, then incubated at 40°C till uniform curdling, when the pH reaches 4.65. The produced yoghurt was cooled overnight at 5± 1°C. In the other step, the drinking yoghurt treatments were manufactured as follows:

- To get 6% sugar in the drinking yoghurt, 50% sterilized distilled water was blended with plain yoghurt and sugared with 12% sugar. This treatment was the control.
- Drinking yoghurt fortified with 3% AECFP: yoghurt was blended with 44% sterilized distilled water sugared with 12% sugar and 6% pasteurized AECFP sugared with 12% sugar (to reach 6% sugar and 3% AECFP in the drinking yoghurt).
- Drinking yoghurt fortified with 6% AECFP: yoghurt was blended with 38% sterilized distilled water sugared with 12% sugar and 12% pasteurized AECFP sugared with 12% sugar (to reach 6% sugar and 6% AECFP in the drinking yoghurt).
- Drinking yoghurt fortified with 9% AECFP: yoghurt was blended with 32% sterilized distilled water sugared with 12% sugar and 18% pasteurized AECFP sugared with 12% sugar (to reach 6% sugar and 9% AECFP in the drinking yoghurt). The prepared drinking yoghurt samples were packed in 250-g glass bottles and then saved in the refrigerator till used to feed the rats. The drinking yoghurt samples were analyzed on the first day.

Chemical analysis

The moisture, total solids, protein, fat, ash and crude fiber contents, as well as the titratable acidity% for buffalo skim milk, AECFP and drinking yoghurt treatments were determined using AOAC (2000) methodology. The pH value of samples was measured by using a laboratory pH meter (type HANNA, Model 8417, Italy). The content of total phenols, flavonoids and antioxidant activity of AECFP were estimated using Batista *et al.* (2011), Ragaei *et al.* (2006) and Maksimovi *et al.* (2005), respectively. HPLC was used in separating and identifying some of polyphenolic compounds according to Goupy *et al.* (1999).

Color measurement of drinking yoghurt

Color measurement (L^* , a^* and b^*) of all drinking yoghurt samples was performed using a Hunter lab color analyzer (Hunterlab Color Flex EZ, USA).

Sensory evaluation

The drinking yoghurt quality was judged through the sensory evaluation by presenting the samples to a panel of ten professional members from the Faculty of Agriculture, Zagazig University. The panelists were asked to judge the samples for color (out of 10 points), flavor (out of 45 points), body and texture (out of 30 points), appearance (out of 15 points) and overall acceptability (out of 100 points) according to Pal and Gupta (1985).

Biological experiment design

Thirty albino male rats weighing 120 ± 10 g were employed in the current investigation. Rats were retained in normal and idealized conditions of cages, temperature and humidity degree, cleaning and daily checking, in the animal house of the Faculty of Agriculture, Zagazig University. Rats were fed on the basal diet (pre-prepared using Campbell, 1963 and Reeves *et al.*, 1993 methods) for two weeks. After an acclimatization period, rats were divided into two groups. Six rats were used in the first group, and represented a negative control group. Twenty-four rats were inoculated with cadmium chloride at a rate of 7 mg/kg BW/day (intraperitoneal) for two weeks to induce hepatotoxicity according to a method of Gali *et al.* (2023). The hepatotoxicity rats were distributed to four groups. One of them used as a positive control group did not receive any treatment. The three groups received 5% w/w from drinking yoghurt fortified with 0, 3 and 9% AECFP daily for four weeks. During the treat period, the amount of food consumed daily and waste was recorded to estimate the food intake. Also, the body weight was

recorded weekly to calculate body weight gain (B.W.G.%) and Food efficiency ratio (F.E.R) according to **Chapman *et al.* (1959)** and **Lee & Nieman (1996)**, respectively.

Biological analysis

At the end of the biological trial, the rats fasted overnight before being euthanized. Blood samples were compiled from the aorta via Wassermann and EDTA tubes and then centrifuged at 4000 rpm for 10 min to separate serum and plasma for conducting chemical analysis. Also, the liver and kidney tissues of all rats were excised, weighed, and then classified into two parts; the first part was homogenized with saline and used to determine antioxidant biomarkers. The second part was washed in saline and immediately fixed into a 10% formalin solution for histological examination.

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, total bilirubin, albumin, globulin, urea and creatinine were assessed using biochemical assays as mentioned in **Young (2001)**. Lipid profile parameters (total cholesterol, HDL-c, LDL-c, VLDL-c and triglycerides) were measured by the methods of **Megraw *et al.* (1979)**, **Young (2001)**, **Burstein *et al.* (1970)**, **Johnson *et al.* (1997)** and **Friedewald *et al.* (1972)**. Antioxidant enzymes levels in liver tissues for all rat groups as malondialdehyde (MDA) (**Sun *et al.*, 1988**), superoxide dismutase (SOD) (**Nishikimi *et al.*, 1972**) and catalase (CAT) (**Aebi, 1984**) were determined.

Histopathological examination

Liver and kidney tissues were fixed in formalin, sectioned, and stained for microscopic evaluation to assess histological changes, according to **Bancroft and Stevens (2013)** technique.

Statistical analysis

The obtained data of all sample analyses (three repetitions) were assessed statistically using the Statistix software program, version 10 (**Statistix, 2013**). A variance analysis (ANOVA) was applied to determine differences between the results of treatments.

RESULTS AND DISCUSSION

Approximate composition of buffalo skim milk and AECFP

Table (1) displays the chemical composition of buffalo skim milk and AECFP. Buffalo skim milk contained 9.78% total solids, 4.00% protein, 0.11% fat and 0.71% ash. AECFP contained 19.20% total solids, 3.10% protein, 0.55% fat, 1.48% ash and 2.84% fiber. The protein and carbohydrate content in fresh *Cassia fistula* fruit was 19.94 and 26.30%, respectively (**Nyeem *et al.*, 2017**). While that content in the powder of *Cassia fistula* seeds was 26 and 50%, respectively making it a good source of nutrients, and thus can be used in food fortification (**Akinyede and Amoo, 2009**). Also, **Algharib *et al.* (2021)** and **Kdam *et al.* (2022)** measured the moisture, lipid, protein, carbohydrates, ash and fiber percentages and found that they represented 10.79 and 3.20%, 2.11 and 3.06%, 17.10 and 16.62%, 59.53 and 64.72%, 4.95 and 4.94%, 5.51 and 7.48 % in *Cassia fistula* seeds and pods respectively.

Table (1): Approximate chemical composition (%) of buffalo skim milk and aqueous extract of *Cassia fistula* pods

Items	Buffalo skim milk	Aqueous extract of <i>Cassia fistula</i> pods
Total solids	9.78± 1.16	19.20± 1.69
Protein	4.00± 0.15	3.10± 0.04
Fat	0.11± 0.30	0.55± 0.13
Ash	0.71± 0.01	1.48± 0.01
Crude fiber	-	2.84± 0.01

Polyphenol compounds of AECFP

Table (2) shows total phenolics, flavonoids, antioxidant activity and the identification of polyphenol compounds present in AECFP. Total phenolics, flavonoids and antioxidant activity of AECFP were 198.61 mg GAE g⁻¹, 91.92 mg QE g⁻¹ and 81.39%, respectively. AECFP has higher levels of total phenolics and flavonoids than the methanolic extract of *Cassia fistula* seed (109.00 mg GAE g⁻¹ and 67.78 mg QE g⁻¹, respectively) and pulp (114.23 mg GAE g⁻¹ and 74.65 mg QE g⁻¹, respectively) (Irshad *et al.*, 2012). Furthermore, these results were in agreement with the studies that exhibited *Cassia fistula* had a high polyphenolic and flavonoid content (Hazra *et al.*, 2022 and Omer *et al.*, 2022). As Jothy *et al.* (2011) and Bargah & Kushwaha (2017) mentioned the aqueous and ethanolic *Cassia fistula* extracts showed high antioxidant activity by inhibiting DPPH and hydroxyl radicals. As shown in the same table, the concentration of polyphenol compounds in *Cassia fistula* pods extract ranged from 17.96 to 2085.88 µg/g. Ferulic acid and ellagic acid were the predominant compounds in *Cassia fistula* pods extract, while caffeic acid was the lowest. In a study by Kashiwada *et al.* (1996), flavan-3-ols and proanthocyanidins like catechin, procyanidin B-2, epicatechin and epiafzelechin are observed in the *Cassia fistula* pods extract. Kaur *et al.* (2019) detected three major polyphenol compounds in ethyl acetate *Cassia fistula* extract using HPLC: catechin, chlorogenic acid and epicatechin with levels of 267.4, 208.5 and 115.8 ppm, respectively. Hence, extracts from *Cassia fistula* may be utilized in the pharmaceutical industry, as a possible nutritional supplement, and as a readily available source of natural antioxidants.

Table (2): Total phenolics, flavonoids, antioxidant activity and identification of polyphenol compounds of aqueous *Cassia fistula* pods extract

Item		Aqueous <i>Cassia fistula</i> pods extract
Total Phenolic (mg GAE g ⁻¹)		198.61± 1.72
Total flavonoids (mg QE g ⁻¹)		91.92± 0.71
Antioxidant activity %		81.39± 1.06
Polyphenol compounds Conc. (µg/g)	Gallic acid	287.07
	Chlorogenic acid	278.31
	Catechin	116.92
	Methyl gallate	367.06
	Caffeic acid	17.96
	Syringic acid	145.24
	Pyro catechol	54.57
	Rutin	1252.17
	Ellagic acid	1963.98
	Vanillin	210.10
	Ferulic acid	2085.88

	Naringenin	357.28
	Daidzein	27.54
	Quercetin	86.47
	Cinnamic acid	43.91

Chemical composition, pH value and titratable acidity of treatments

The composition of drinking yoghurt fortified with AECFP is exposed in Table (3). The moisture content decreased with increasing the AECFP added ratio. This decrease was significant between the control of drinking yoghurt and that fortified with 9% AECFP, and insignificant between the control and drinking yoghurt fortified with 3 and 6% AECFP. Decreased moisture content can be attributed to the rise of total solids in AECFP compared to buffalo skim milk.

The protein content increased with increasing AECFP ratio in drinking yoghurt. This increase was significant between the yoghurt control and yoghurt fortified with 6 and 9% AECFP, and insignificant between the yoghurt control and yoghurt fortified with 3 and 6% AECFP.

Furthermore, the control of drinking yoghurt had lower fat and ash content. The drinking yoghurt fortified with 6 and 9% AECFP was significantly higher than the control. The rise in ash content may be due to the mineral content in AECFP, where ash content represents the total mineral content, and higher levels of AECFP introduce more minerals into the yoghurt. This is consistent with **Ghanimah *et al.* (2024)**, who demonstrated that additives with higher mineral content increase the ash content of dairy products.

The same table indicates that the gradual increase of AECFP led to significant decreases in pH values of drinking yoghurt fortified with 6 and 9% AECFP compared with the control. In contrast, the acidity was increased slightly with the rise of AECFP in drinking yoghurt. This suggests that while AECFP influences acidity, its effect is relatively moderate, this may be due to the small ratio of the aqueous extract added to drinking yoghurt (**Al-Soudy *et al.*, 2020**). The relationship between pH and acidity is also documented, with lower pH usually corresponding to higher acidity (**Sadler and Murphy 2010**).

Table (3): Chemical composition, pH value and titratable acidity of drinking yoghurt fortified with aqueous extract of *Cassia fistula* pods

Items	Yoghurt control	Yoghurt + 3% AECFP	Yoghurt + 6% AECFP	Yoghurt + 9% AECFP	LSD
Moisture %	90.65± 0.82 ^a	89.85± 0.96 ^{ab}	88.67± 1.73 ^{ab}	87.86± 1.07 ^b	2.26
Protein %	1.73± 0.06 ^b	1.95± 0.15 ^{ab}	2.09± 0.11 ^a	2.21± 0.20 ^a	0.26
Fat %	0.04± 0.01 ^c	0.05± 0.01 ^{bc}	0.08± 0.02 ^b	0.12± 0.03 ^a	0.03
Ash %	0.34± 0.01 ^d	0.37± 0.02 ^c	0.47± 0.01 ^b	0.56± 0.01 ^a	0.02
pH value	5.17± 0.31 ^a	4.91± 0.17 ^{ab}	4.74± 0.07 ^b	4.65± 0.05 ^b	0.35
Acidity %	0.45± 0.02 ^a	0.47± 0.04 ^a	0.48± 0.02 ^a	0.50± 0.03 ^a	0.05

AECFP: aqueous extract of *Cassia fistula* pods.

Different letters put on mean values in the same row are considered significantly different.

Color measurement results

Table (4) shows that the L^* value, representing lightness, decreased significantly with increasing the AECFP ratio in drinking yoghurt. This indicates that the yoghurt becomes darker as more AECFP is added, it almost looks like a chocolate milk color. These results align with

McGuire (2012) who reported that the addition of colored components will reduce the lightness of dairy products whiteness. The a^* value, indicating the redness, increased from the negative values in the control sample to positive values in drinking yoghurt samples fortified with AECFP. The highest significant increase in a^* values was recorded for drinking yoghurt fortified with 9% followed by 6% AECFP compared with control. The highest b^* value, representing the yellowness, was observed in yoghurt fortified with 3% AECFP, and it decreases slightly with a higher AECFP ratio. This suggests that while AECFP initially increases yellow coloration, its effect may plateau or slightly diminish with higher concentrations. This behavior may be attributed to the color characteristics of AECFP, which may contain varying levels of yellow pigments. These results align with **Abdeldaiem and Mokbel (2022)**, who found that the L^* value was significantly decreased, but a^* and b^* values were increased in parallel with tamarind extracts increased in yoghurt drinks.

Table (4): Color of fresh drinking yoghurt

Items	Yoghurt control	Yoghurt + 3% AECFP	Yoghurt + 6% AECFP	Yoghurt + 9% AECFP	LSD
L^*	80.90± 0.12 ^a	44.33± 0.13 ^b	33.26± 0.72 ^c	28.39± 0.15 ^d	0.71
a^*	-4.07± 0.01 ^c	4.09± 0.05 ^b	4.71± 1.15 ^{ab}	5.70± 0.03 ^a	1.08
b^*	7.24± 0.06 ^d	14.17± 0.07 ^a	12.97± 0.24 ^b	11.85± 0.05 ^c	0.24

L^* : (Lightness), a^* : (redness), b^* : (yellowness), AECFP: aqueous extract of *Cassia fistula* pods. Different letters put on mean values in the same row are considered significantly different.

Sensory properties of fresh drinking yoghurt

The results presented in Table (5) showed that the color and appearance scores were increased with a higher AECFP ratio in drinking yoghurt, with significant improvements observed at 6% and 9% AECFP. This suggests that higher AECFP levels enhance the color perception of yoghurt, making it more appealing to consumers. AECFP may contribute positively to the color due to its pigments (**McGuire, 2012**). The highest scores at 9% AECFP reflect a more favorable color attribute, enhancing visual appeal to consumers. Flavor scores were significantly higher of drinking yoghurt fortified with 6% AECFP compared to the control and 3% AECFP. The 9% AECFP yoghurt also has a high flavor score, though slightly lower than at 6% AECFP, but without any significant differences. The body and texture scores were improved significantly with increasing AECFP ratio in yoghurt, particularly notable at 9% AECFP. This indicates that AECFP positively affects the consistency of the yoghurt, making it thicker than the control. The increase in scores with AECFP addition is consistent with findings that certain additives can enhance the textural attributes of dairy products (**Salehi, 2021**). Total acceptability scores of prepared drinking yoghurt showed a significant increase with a rise of the AECFP ratio, peaking at 9% AECFP. This is due to enhancements in color, flavor, body texture and appearance, reflecting the positive impact of AECFP on the overall sensory quality of the yoghurt (**Bayarri et al., 2011**). These results are consistent with a study of **Shahein et al. (2022)**, who noticed a significant amelioration in the sensory properties of drinking yoghurt after fortifying it with golden berry juice. Moreover, **Abdeldaiem and Mokbel (2022)** established that the fortification of the yoghurt drink with 5 and 7% tamarind extract significantly improved its sensory properties.

Table (5): Sensory properties of fresh drinking yoghurt

Items	Yoghurt control	Yoghurt + 3% AECFP	Yoghurt + 6% AECFP	Yoghurt + 9% AECFP	LSD
Color (10)	7.80± 0.84 ^b	8.20± 1.30 ^{ab}	9.00± 0.71 ^a	9.20± 0.27 ^a	1.16
Flavor (45)	38.20± 2.28 ^b	39.20± 2.59 ^b	43.00± 1.22 ^a	42.60± 1.82 ^a	3.53
Body and texture (30)	20.60± 1.82 ^c	20.80± 1.30 ^c	25.80± 2.39 ^b	29.20± 1.10 ^a	2.31
Appearance (15)	11.40± 1.52 ^b	11.60± 1.14 ^b	13.00± 1.41 ^{ab}	14.20± 0.84 ^a	1.63
Total acceptability (100)	78.00± 2.83 ^c	79.80± 2.77 ^c	90.80± 1.92 ^b	95.20± 1.15 ^a	4.08

AECFP: aqueous extract of *Cassia fistula* pods.

Different letters put on mean values in the same row are considered significantly different.

Body weight gain, food intake and FFR of hepatotoxic rats

Impact of drinking yoghurt fortified with aqueous extract of *Cassia fistula* pods intake on body weight gain, food intake and FFR of hepatotoxic rats is offered in Table (6). The lowest mean values of body weight gain, body weight gain %, food intake and FFR were recorded in the control (+) group (hepatotoxic rats). There was a significant reduction in mean values of body weight gain and FFR for all rat groups compared with the control (-) rats group (normal rats). When hepatotoxic rats received drinking yoghurt, the data of body weight gain, food intake and FFR improved significantly especially when hepatotoxic rats received drinking yoghurt fortified with AECFP compared with control (+) rats. These results agree with authors who stated that cadmium chloride induced an abnormal rapid reduction in body weight gain in hepatotoxic rats (**Layachi and Kechrid, 2012 and Saedi *et al.*, 2020**). The decrease in body weight gain may be due to the reduction in food intake and the overall increased deterioration of proteins and lipids as a result of cadmium toxicity (**Layachi and Kechrid, 2012**). Using the drinking yoghurt and fortified with AECFP in the feeding of hepatotoxic rats led to an improvement in body weight gain, body weight gain %, food intake and FFR. The reason for this improvement is that both drinking yoghurt and yoghurt fortified with AECFP contain many biologically active compounds and some essential nutrients that have a significant and effective effect on weight gain.

Table (6): Impact of drinking yoghurt fortified with aqueous extract of *Cassia fistula* pods intake on mean values of body weight gain, food intake and FFR of hepatotoxic rats

Parameters	Groups					LSD
	Control (-) rats	Hepatotoxic rats Control (+)	Hepatotoxic rats + Yoghurt control 0% AECFP	Hepatotoxic rats + Yoghurt 3% AECFP	Hepatotoxic rats + Yoghurt 9% AECFP	
Body weight gain (g/ 30 days)	60.00±3.61 ^a	28.67±2.08 ^e	33.33±1.15 ^d	40.00±2.65 ^c	47.67±0.58 ^b	4.15
Body weight gain %	23.89±2.48 ^a	12.93±1.84 ^c	14.53±1.07 ^{bc}	17.32±1.46 ^b	21.48±1.07 ^a	3.04
Food intake (g / day)	26.63±1.20 ^a	23.90±0.94 ^b	24.37±1.54 ^b	25.05±0.85 ^{ab}	26.39±0.36 ^a	1.92
Food intake (g / 30 days)	798.90±35.94 ^a	716.90±28.30 ^b	731.00±46.23 ^b	771.50±32.24 ^{ab}	791.70±10.80 ^a	59.71
FER	0.075±0.001 ^a	0.040±0.005 ^d	0.046±0.002 ^d	0.052±0.005 ^c	0.060±0.001 ^b	0.006

AECFP: aqueous extract of *Cassia fistula* pods.

Different letters put on mean values in the same row are considered significantly different.

Impact of drinking yoghurt on liver and kidney weights

As presented in Table (7), the mean organ weights in hepatotoxic rats who received only the basal diet (control +) were significantly less than those in other groups. When the hepatotoxic rat groups received drinking yoghurt, the liver and kidney weights were significantly increased. Moreover, no significant differences were found between hepatotoxic rats receiving yoghurt fortified with 9 % AECFP and the control (-) rats group. These results are harmonious with **Gathwan *et al.* (2012)** and **Dwivedi (2021)** who indicated that cadmium chloride induced necrotic effect in the liver led to apoptotic and decreased liver weight.

Table (7): Impact of drinking yoghurt fortified with aqueous extract of *Cassia fistula* pods intake on liver and kidney weights of hepatotoxic rats

Parameters	Groups					LSD
	Control (-) rats	Hepatotoxic rats Control (+)	Hepatotoxic rats + Yoghurt control 0% AECFP	Hepatotoxic rats + Yoghurt 3% AECFP	Hepatotoxic rats + Yoghurt 9% AECFP	
Liver weight	9.16±1.06 ^a	6.82±0.95 ^c	7.77±1.05 ^{bc}	8.32±0.70 ^{ab}	8.46±0.88 ^{ab}	1.20
Kidney weight	2.07±0.09 ^a	1.60±0.04 ^d	1.81±0.04 ^c	1.88±0.05 ^{bc}	1.99±0.07 ^{ab}	0.11

AECFP: aqueous extract of *Cassia fistula* pods.

Different letters put on mean values in the same row are considered significantly different.

Impact of drinking yoghurt on liver function

The liver function analysis (Table 8) exposed a significant increase in ALT, AST, ALP, total protein, total bilirubin, albumin and globulin of control (+) rats, and a significant reduction in hepatotoxic rats receiving drinking yoghurt, especially yoghurt fortified with AECFP. No substantial differences were noticed between the control (-) rats and hepatotoxic rats receiving yoghurt fortified with 9 % AECFP in ALT, total bilirubin and globulin levels.

According to **Yogalakshmi *et al.* (2010)** and **Sivaraj *et al.* (2011)** studies, elevated liver enzyme levels indicate cellular leakage and a lack of functional integrity of the hepatic membrane architecture, which causes the enzymes to be released into the bloodstream from the cell cytosol. Also, elevated bilirubin level denotes considerable hepatocellular injury. The pre-treatment with *Cassia fistula* pod extract decreased the elevations in serum AST, ALT, ALP, and total bilirubin levels caused by carbon tetrachloride (CCl₄), as reported by **Sharma *et al.* (2016)**. The therapeutic effects of *Cassia fistula* pod extract may be due to its high content of antioxidant compounds that remedy membrane damage and oxidative stress. Consequently, a significant decrease in liver function parameters occurs (**Kumar *et al.*, 2017**)

These results are consistent with the research of **Tariq *et al.* (2024)** who established that *Cassia fistula* pods extract administration induced a reduction in liver enzymes (ALT and AST levels), This attributed to its antioxidant compounds, which confirm efficiency of the extract in conserving the normal functional state of the liver.

Table (8): Impact of drinking yoghurt fortified with aqueous extract of *Cassia fistula* pods intake on liver function of hepatotoxic rats

Parameters	Groups					LSD
	Control (-) rats	Hepatotoxic rats Control (+)	Hepatotoxic rats + Yoghurt control 0% AECFP	Hepatotoxic rats + Yoghurt 3% AECFP	Hepatotoxic rats + Yoghurt 9% AECFP	
ALT (u/l)	23.92±1.96 ^d	51.72±2.65 ^a	41.76±0.73 ^b	37.25±1.62 ^c	26.53±2.25 ^d	3.55
AST (u/l)	90.61±5.01 ^e	179.23±6.61 ^a	134.50±2.95 ^b	124.57±3.64 ^c	106.66±6.86 ^d	9.55
ALP (u/l)	90.57±6.87 ^d	167.37±7.36 ^a	135.40±2.50 ^b	127.27±1.94 ^b	106.17±4.81 ^c	9.44
Total protein (mg /dl)	6.01±0.56 ^d	9.92±0.18 ^a	8.26±0.11 ^b	7.86±0.27 ^b	7.07±0.17 ^c	0.55
Total bilirubin (mg /dl)	0.52±0.03 ^d	1.28±0.07 ^a	0.83±0.02 ^b	0.74±0.04 ^c	0.57±0.03 ^d	0.07
Albumin (mg /dl)	3.13±0.35 ^d	6.74±0.39 ^a	4.65±0.14 ^b	4.35±0.03 ^{bc}	3.97±0.15 ^c	0.46
Globulin (mg /dl)	2.80±0.28 ^c	4.19±0.13 ^a	3.90±0.08 ^a	3.35±0.17 ^b	3.07±0.07 ^{bc}	0.30

AECFP: aqueous extract of *Cassia fistula* pods.

Different letters put on mean values in the same row are considered significantly different.

Impact of drinking yoghurt on kidney function

The impact of drinking yoghurt on the kidney function of hepatotoxic rats is presented in Table (9). It could be noted that the higher urea and creatinine levels were recorded for control (+) rats, while the lower levels were recorded for control (-) rats with significant differences. The reason for this may be ascribed to the direct impact of cadmium on renal tubules and resulting oxidative stress and nephrotoxicity (Golbaghi *et al.*, 2019). It has been shown that the proximal tubule cells' transporters, metabolizing enzymes, and cadmium sensors allow for the reabsorption of cadmium, which is precipitated in these cells. cadmium can displace other metals from metalloproteins after it reaches cells, which can impact kidney function (Adel and Ghalwash, 2018). When hepatotoxic rats received drinking yoghurt, especially that fortified with 3 and 9% AECFP, the urea and creatinine levels significantly decreased. This is consistent with what was stated by researcher Kumar *et al.* (2017) that *Cassia fistula* bark extract had a significant anti-inflammatory effect against lipid peroxidation and free radical generation in liver and kidney homogenates.

Table (9): Impact of drinking yoghurt fortified with aqueous extract of *Cassia fistula* pods intake on kidney function of hepatotoxic rats

Parameters	Groups					LSD
	Control (-) rats	Hepatotoxic rats Control (+)	Hepatotoxic rats + Yoghurt control 0% AECFP	Hepatotoxic rats + Yoghurt 3% AECFP	Hepatotoxic rats + Yoghurt 9% AECFP	
Urea (mg / dl)	19.90±1.73 ^e	37.53±0.72 ^a	28.43±0.84 ^b	25.97±0.81 ^c	22.07±1.05 ^d	1.99
Creatinine (mg / dl)	0.56±0.02 ^e	1.16±0.03 ^a	0.91±0.04 ^b	0.81±0.04 ^c	0.65±0.02 ^d	0.05

AECFP: aqueous extract of *Cassia fistula* pods.

Different letters put on mean values in the same row are considered significantly different.

Impact of drinking yoghurt on lipid profile

The results of the lipid profile are displayed in Table (10). Highly significant increases in the levels of triglyceride and total cholesterol were recorded in control (+) followed by hepatotoxic rats receiving drinking yoghurt control, then rats receiving yoghurt fortified with 3% AECFP. Conversely, high significant decreases in triglyceride and total cholesterol levels were observed

in control (-) rats followed by hepatotoxic rats received yoghurt fortified with 9% AECFP. Also, there was a significant reduction in levels of LDL-c and VLDL-c in hepatotoxic rats received drinking yoghurt and yoghurt fortified with AECFP when compared with control (+) rats. Moreover, there was a significant increase in HDL-c levels of hepatotoxic rats received drinking yoghurt and yoghurt fortified with AECFP when compared with control (+) rats. While no significant differences were noted in HDL-c levels of hepatotoxic rats receiving yoghurt fortified with 3 and 9% AECFP and control (-) rats. This is ascribed to *Cassia fistula* extract efficiency in modulating lipid metabolism and exerting antioxidant effects by its polyphenols content that supported hepatic LDL receptor binding facilitated led to LDL levels reduction (**Tariq *et al.*, 2024**). While the rise in HDL levels may resulted from improved lecithin-cholesterol acyltransferase activity, stimulating reverse cholesterol transport and endothelial protection (**Ossoli *et al.*, 2019**). Likewise, the study of **Sharma *et al.* (2016)** exhibited that pre-treating the liver with a hydro-alcoholic extract of *Cassia fistula* pods proved effective in protecting the liver's cholesterol metabolism from disruptions caused by CCl₄.

Table (10): Impact of drinking yoghurt fortified with aqueous extract of *Cassia fistula* pods intake on lipid profile of hepatotoxic rats

Parameters	Groups					LSD
	Control (-) rats	Hepatotoxic rats Control (+)	Hepatotoxic rats + Yoghurt control 0% AECFP	Hepatotoxic rats + Yoghurt 3% AECFP	Hepatotoxic rats + Yoghurt 9% AECFP	
Triglyceride (mg /dl)	75.25±8.02 ^c	191.97±6.16 ^a	153.30±3.32 ^b	134.97±1.96 ^c	91.90±3.58 ^d	9.28
Total cholesterol (mg /dl)	100.26±4.20 ^d	182.03±5.08 ^a	135.63±3.26 ^b	133.50±2.04 ^b	112.80±3.70 ^c	6.91
HDL-c (mg /dl)	62.36±3.43 ^{ab}	43.37±3.97 ^d	54.23±3.96 ^c	57.80±2.29 ^{bc}	66.04±1.51 ^a	5.79
LDL-c (mg /dl)	22.85±2.57 ^d	100.27±2.03 ^a	50.62±1.39 ^b	49.38±1.53 ^b	28.38±2.89 ^c	3.93
VLDL-c (mg /dl)	15.05±1.60 ^d	38.39±1.23 ^a	26.66±0.66 ^b	27.01±0.40 ^b	18.38±0.72 ^c	1.85

AECFP: aqueous extract of *Cassia fistula* pods.

Different letters put on mean values in the same row are considered significantly different.

Impact of drinking yoghurt on antioxidant enzymes

The results in Table (11) show a significant increase in MAD level and a significant decrease in CAT and SOD levels in control (+) rats compared with control (-) rats. Oxidative stress resulting from cadmium toxicity could be to blame for this, as it raises ROS and hydrogen peroxide levels, which in turn decreases the activity and levels of antioxidant enzymes like CAT, SOD, and Gpx and increases MDA (**Branca *et al.*, 2020 and Souza-Arroyo *et al.*, 2022**). When hepatotoxic rats received yoghurt control and yoghurt fortified with AECFP, the levels of MAD decreased and CAT and SOD increased. This reduction and increase were significant compared with control (+) rats. This may be due to the high phenolic and flavonoid content of *Cassia fistula* pods, thus their antioxidant activity (**Maheep *et al.*, 2010 and Sharma *et al.*, 2016**). These results match with **Kaur *et al.* (2019)** who concluded that ethyl acetate extract of *Cassia fistula* had notable hepatoprotective potential by reducing oxidative stress by modulation of antioxidant enzymes.

Table (11): Impact of drinking yoghurt fortified with aqueous extract of *Cassia fistula* pods intake on MDA, CAT and SOD levels of hepatotoxic rats

Parameters	Groups					LSD
	Control (-) rats	Hepatotoxic rats Control (+)	Hepatotoxic rats + Yoghurt control 0% AECFP	Hepatotoxic rats + Yoghurt 3% AECFP	Hepatotoxic rats + Yoghurt 9% AECFP	
MAD (nmol/ L)	11.23±0.86 ^c	52.75±4.72 ^a	32.07±2.63 ^b	28.08±0.64 ^b	13.28±0.90 ^c	4.54
CAT (u/ml)	97.28±4.39 ^a	33.17±2.14 ^e	50.87±2.19 ^d	71.27±1.97 ^c	85.67±2.50 ^b	5.07
SOD (u/ml)	131.44±4.15 ^a	32.07±2.74 ^e	60.00±2.30 ^d	77.83±6.15 ^c	111.17±7.52 ^b	9.07

AECFP: aqueous extract of *Cassia fistula* pods.

Different letters put on mean values in the same row are considered significantly different.

Histopathological results

The results of histological examination of the liver and kidney tissues for normal and hepatotoxic rat groups are exposed in Fig. (1 and 2).

Liver

Fig. (1. A) appears section from the liver tissues of the control (-) group which demonstrates the normal histological structure of the central vein (C.V.) with regular hepatic cords (thick arrow) that are separated by blood sinusoids (arrowhead). The hepatic cords contain normal hepatocytes with central, spherical, and lightly stained nuclei (wave arrow). Whereas Fig. (1. B) shows the control (+) group (hepatotoxic rats), which appearances a dilated central vein (C.V.) (wave arrow) surrounded by irregular hepatic cords (cube) that are separated by dilated and congested (thick arrow) blood sinusoids. The Fig. (1. C) of the control (+) group shows revealing moderate hydropic degeneration of hepatocytes (thick arrows). When hepatotoxic rats received yoghurt (Fig. 1. D), the treated hepatotoxic rats exhibited remarkably decreased histopathological changes than observed in control (+) rats. The hepatotoxic rats received yoghurt control demonstrate nearly normal central vein (C.V.) and blood sinusoids (arrowhead). Some hepatocytes appear nearly normal (wave arrow), while others show mild vacuolar degeneration (thick arrow). Fig. (1. E) of hepatotoxic rats received yoghurt fortified with 3% AECFP, shows restoration of most histological architecture (wave arrow) except dilatation and congestion of blood sinusoids (thick arrow) as well as few degenerated hepatic cords (cube). While hepatotoxic rats received yoghurt fortified with 9% AECFP represents a marked improvement in hepatic parenchyma ((Fig. 1. F). Moreover, the hepatic sinusoids appear less congested (wave arrow). The congestion of the central vein (C.V.) is also observed. These results are consistent with **Tariq *et al.* (2024)**, who observed that *C. fistula* extract led to improvements in liver architecture and reduced lipid accumulation, hepatocellular and necrosis. Also, the histopathological results of **Das *et al.* (2008)** and **Sharma *et al.* (2016)** confirmed that the aqueous and ethanolic extracts of *Cassia fistula* pulp had a protective effect on hepatic histoarchitecture of CCl₄ intoxicated rats.

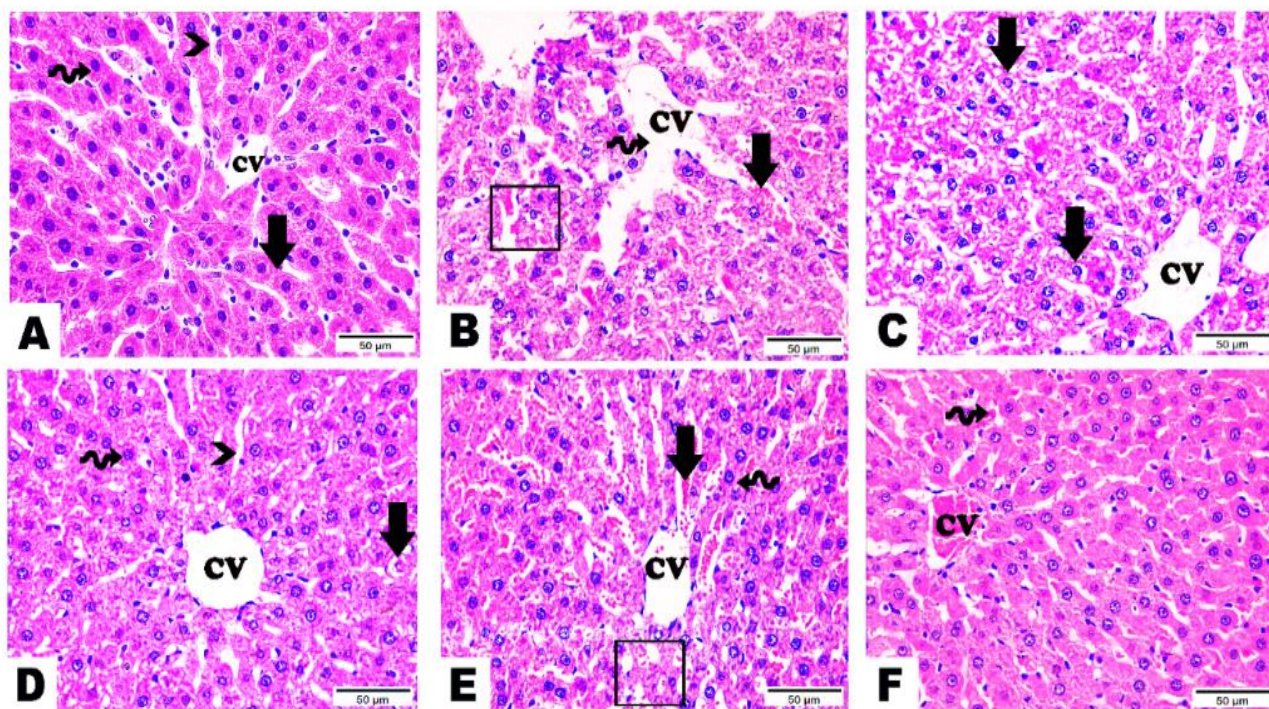


Fig. (1): Photomicrographs of the histopathological variations in liver tissue sections of rats among experimental groups as following: A: Control (-) group, B and C: Control (+) group, D: hepatotoxic rats received yoghurt control, E: hepatotoxic rats received yoghurt fortified with 3% aqueous extract of *Cassia fistula* pods, F: hepatotoxic rats received yoghurt fortified with 9% aqueous extract of *Cassia fistula* pods. (Hematoxylin and Eosin Stain, Magnification Power = x400, Scale bar= 50µm).

Kidney

The histopathological changes of kidney tissue sections between studied groups are seen in Fig. (2), the control (-) group (Fig. 2. A) exhibits the normal histological structure of renal corpuscle (cube), proximal (wave arrow), and distal (thick arrow) convoluted tubules. On the contrary, the control (+) group (Fig. 2. B) shows vacuolation of mesangial cells of renal corpuscles (cube), degeneration of renal tubules (arrowhead), desquamation of tubular epithelium (wave arrow) in addition to the presence of hyaline cast (thick arrow). Some sections of the control (+) rat's renal cortex reveal a loss of renal corpuscle (cube), mild congestion (wave arrow), necrosis of renal tubules (thin arrow), vacuolar degeneration of renal cells with pyknotic nuclei (thick arrow), and desquamated lining epithelium (arrowhead) (Fig. 2. C). These effects in kidney tissues are similar to what mentioned by **Liu *et al.* (2019)** and **Satarug (2024)**, who found that cadmium chloride induces apoptosis, the glomeruli were swelled, renal tubules were lesioned, the epithelial cells became necrotic, renal interstitial congestion and increase inflammatory cells infiltrated, thus cause renal damage. Regarding kidney tissues in hepatotoxic rats that received yoghurt control, it is noted in Fig. (2. D) normal renal cortex and glomerular tufts (cube). The intratubular hyaline cast (thick arrow) and desquamated tubular lining (wave arrow) reduced to a greater extent (remarkably decreased). The hepatotoxic rats that received

yoghurt fortified with 3% AECFP exhibited markedly reduced renal injury with limited renal degeneration, hyalinization (thin arrow), and tubular necrosis (thick arrow) (Fig. 2. E). Whereas, the hepatotoxic rats that received yoghurt fortified with 9% AECFP represents a normal histological architecture of the renal cortex except for some areas highlighted with hyaline cast in one tubule (wave arrow), few degenerations (arrowhead), and desquamation of lining epithelium (thick arrow), as is clear in the Fig. (2. F). These results are matched with **Kumar *et al.* (2017)**.

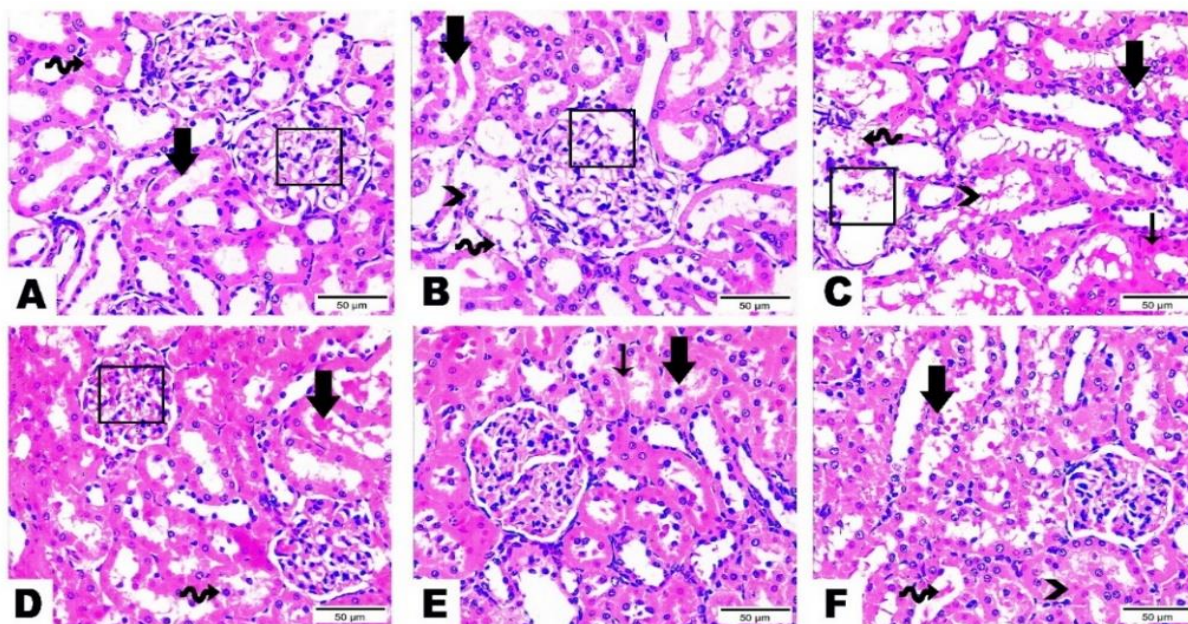


Fig. (2): Photomicrographs of the histopathological variations in kidney tissue sections of rats among experimental groups as following: A: Control (-) group, B and C: Control (+) group, D: hepatotoxic rats received yoghurt control, E: hepatotoxic rats received yoghurt fortified with 3% aqueous extract of *Cassia fistula* pods, F: hepatotoxic rats received yoghurt fortified with 9% aqueous extract of *Cassia fistula* pods. (Hematoxylin and Eosin Stain, Magnification Power = x400, Scale bar= 50µm).

CONCLUSION

Based on the present study, it can be concluded that the aqueous extract of *Cassia fistula* pods (AECFP) had high antioxidant activity and high contents of total phenolic and flavonoids. The fortifying drinking yoghurt with AECFP not only enhances its nutritional and sensory qualities but also improved the biochemical markers and lipid profiles and up regulates the antioxidant enzymes MAD, CAT and SOD of hepatotoxic rat blood. Also, it led to improvements in hepatic histoarchitecture. Thus, it can be recommended to add AECFP as a natural ingredient in the manufacture of functional drinking yoghurt and use it to alleviate the symptoms of liver damage.

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