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Effect of processed cheese supplemented with spirulina against carbon tetrachloride (CCl_4)-induced liver damage and nephrotoxicity in rats

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ABSTRACT

The present study was performed to evaluate the possible effect of processed cheese supplemented with spirulina against carbon tetrachloride (CCl_4)-induced liver damage in rats. Thirty-six of Albino rats were randomly divided into six experimental groups.The first group control (ve-) consists of 6 rats fed on standard diet for 8 weeks. The second group (injected rats) consists of 30 rats injected with Carbon tetrachlorideCCl_4 in paraffin oil (50%v/v) at the level of 2 ml/kg of body weight twice per week by subcutaneous injection for two weeks and then divided into five subgroups of six rats each and fed different proportions of spirulina powder added to processed cheese for 8 weeks. These were divided into control (ve +) group fed only the standard diet, processed cheese only; processed cheese with spirulina powder (2%) group; processed cheese with spirulina powder (4%) group and processed cheese with spirulina powder (6%) group.

The current results showed significant decrease ($p \leq 0.05$) in the levels of the biochemical parameters of liver and kidney functions and there was a significant reduction in lipid profile. The histopathological investigation of liver and kidney in rats injected with CCl_4 and fed on processed cheese supplemented with spirulina had been improved than control (ve+) group.

***Keywords*:** Processed cheese, spirulina, antioxidant, liver, CCl_4.

INTRODUCTION

Processed cheese is a very popular product especially for children due to its pleasant flavor and distinctive texture. It differs from natural cheese where it is not made directly from milk, however, its main ingredient is natural cheese. It is produced by blending natural cheeses of different ages and degrees of maturity in the presence of emulsifying salts and other dairy and nondairy ingredients, followed by heating and continuous mixing to form a homogeneous product with an extended shelf life (Guinee *et al*., 2004). The initial idea of processed cheese was to increase the shelf life of natural cheese and find alternative uses for natural cheese that was difficult to sell. In recent times, there was an interest in the development of processed cheese in order to raise its nutritional value to benefit people who suffer from diseases such as heart disease or hardening of the arteries, reduce the cost of production or increase the shelf life. In the United States, the development of processed cheese was brought about by Kraft in 1916, when he preserved natural cheese in

cans by heating and mixing it in order to increase its shelf life (Kapoor and Metzger, 2008). Also, new varieties of processed cheese were produced using non-conventional additives, such as the production of processed cheese with peppers, spices, oat and olives and smoked processed cheese etc. The nutritional and potential therapeutic value of food is a key characteristic in the development of new value-added products that are manufactured for health-conscious consumers (Fox *et al*., 2017).

Microalgae are known to be rich in proteins, amino acids, vitamins and various minerals, as well as polysaccharides, sterols and polyunsaturated fatty acids as bioactive compounds and pigments as coloring agents with important functional characteristics (Bosnea *et al*., 2020; Durmaz *et al*., 2020). The antioxidant and anti-inflammatory activities of microalgae can significantly contribute to human health. They can activate cellular antioxidant enzymes, inhibit lipid peroxidation and DNA damage, scavenge free radicals, and enhance the activity of superoxide dismutase and catalase (Wu *et al*., 2016). In alignment with the trend towards increased dietary intake and healthy lifestyles, consumers are shifting preferences from artificial additives to natural substances. Natural colors derived from plants, fruits, or animals are extracted and purified, showcasing beneficial biological activities as antioxidants and anticancer agents (Costa *et al*., 2019).

Spirulina, a type of blue-green algae, is a branch of cyanobacteria renowned worldwide as a dietary supplement due to its richness in protein (50-60%), antioxidants, essential fatty acids, etc. (Ayenampudi *et al*., 2023). The potential application of phycocyanin, generally recognized as safe (GRAS), has attracted attention not only in cosmetics and personal care formulations, but also in food products (Mohammadi-Gouraji *et al*., 2019). Phycocyanin is a predominant pigment in Spirulina platensis and possess inhibitory properties against various harmful free radicals such as alkoxyl, hydroxyl, and peroxide, albeit diminishing over time (Romano *et al*., 2000). With their coloring, antioxidant, and antimicrobial properties, phycocyanin finds utility in various food formulations such as yogurt, cheese, ice cream, etc., with their efficacy well established in numerous studies (Safari *et al*., 2022). The potential applications of Spirulina platensis as a nutritional component to enhance the health properties of products like dietary supplements, beverages, fermented sweets, grains, bakery products, desserts, cakes, confectionery products, biscuits, snacks, soups, salad dressings, and dairy products like ice cream, yogurt, and milk-based beverages have been explored (Asadi *et al*., 2020). Despite their inherent instability, derived colorants from Spirulina offer potential health benefits during consumption, functioning as antioxidants and anti-cancer agents. Furthermore, rich in minerals and vitamins, notably potassium, calcium, magnesium, selenium, iron, zinc, and B group vitamins. The water-soluble B group vitamins play pivotal roles in DNA repair, electron transfer, fatty acid synthesis, and one-carbon metabolism (Costa *et al*., 2019).

Spirulina incorporation in the dairy products has become a fairly common practice due to its sensory effects, but also to the functional properties that gives to food. Spirulina offers important properties through its antimicrobial, antioxidant, anticancer and antihypertensive activity, strengthens the immune system, along with other pharmacological effects being included in the category of superfoods (Abdel-Moneim *et al*., 2022; Terpou *et al*., 2020). Spirulina derived bioactive peptide fractions can be applied as nutraceutical ingredients in food and pharmaceuticals (Ovando *et al*., 2018).

The liver is a vital organ admitted in more than five hundred metabolic responses within the biological system and one of its major capacities is the detoxification of injurious substances or poison. However, it can be damaged by toxicants, which within the process may mutilate the metabolic actions of the liver, thus giving rise to liver injury (Zamora *et al.,* 2014; El-Nahrawy *et al.,* 2017). Liver fibrosis is attached to inflammation, tissue remodeling under wound healing and excessive accumulation of extracellular matrix (Neubauer *et al*., 2001).

Carbon tetrachloride (CCl_4) is used as a chemical inducer of liver injury in rats and mice, Also,various studies indicated that CCl_4 can induce kidney damage (Yoshioka*et al*., 2016). Spirulina protects liver tissues from damage by reducing the level of lipid peroxidation and increasing antioxidant activities. Khalil *et al.* (2018) reported the hepatoprotective potential of spirulina in fibrosis and accredited this to the antioxidant effects of spirulina and to its ability to potentiate the antioxidant system. In addition, Spirulina improved activity of the antioxidant system and decreased lipid peroxidation in the liver of cadmium-intoxicated rats (Jeyaprakash and Chinnaswamy, 2005). Moreover, CCl4 induced a significant induction of renal disorder (Ogeturk *et al*., 2005), oxidative damage and DNA fragmentation as evidenced by increased plasma creatinine, urea and uric acid levels, increased lipid peroxidation (malondialdehyde [MDA]) and protein carbonyl (Makni *et al*., 2012).

Various studies were carried out on the protective effects of different materials against CCl_4-induced kidney damages in rats and mice. These include vanillin (Makni*et al*., 2012), Zinc Sulfate (Yoshioka *et al*., 2016), however El-Tantawy (2015), Zheng *et al*. (2018), Kapoor and Mehta (1998) and Abdel-Daim *et al*. (2020) investigated the positive effect of using spirulina for the treatment of liver damage. On the other hand, spirulina supplementation reduced urea levels (El-Sheekh *et al*., 2020), creatinine levels (Khan *et al*. (2019) and uric acid levels (Farooq *et al*. (2021) in rats with drug-induced nephrotoxicity.

Therefore, the aim of these research is to evaluate the effect of processed cheese supplemented with spirulina against carbon tetrachloride (CCl_4)-induced liver and kidney damage in rats.

MATERIALS AND METHODS

Materials

Ras cheese (1-month-old) was obtained from the herd of the dairy cattle at Faculty of agriculture, Cairo university. Skim milk powder made was obtained from the local market (96% T. S, 1.5% milk fat, 34% protein). Algae (Spirulina platensis) in the form of freeze-dried powder was obtained from Al Nada Farms, Giza, Egypt. Joha S9 emulsifying salt was obtained from the BK Giulini Chemie GmbH, Landenberg, Germany. The kits used for analysis were obtained from Bio-diagnostic Co. Dokki, Egypt.Carbon tetrachloride CCl_4 was obtained from Memphis Company for Pharmaceutical and Chemical industries, Cairo, Egypt.

Experimental procedures

Production of Spreadable Processed cheese

Processed cheese spread (PCS) was made using Ras cheese (1-month-old). The PCS formula were adjusted to contain 35% moisture and 46% fat in dry matter, and 2.5% emulsifying salts (Sgs+No (1:1)). The Ras cheese, Spirulina (2,4,6%), water and emulsifying salt were added. Consecutively in a laboratory style processing kettle (Awad, 1996). The mixture was cooked for 8 min. at

85-90ºC using indirect steam at pressure 2-2.5kg/cm^2. The melted processed cheese was purred into wide mouth glass jars and capped directly after filling. The resultant cheese was analyzed when fresh, after 1, 2 and 3 months of storage at refrigerator (5±2ºC).

Animal, housing and diets

Thirty-six of Albino rats weighing about 198±7g was obtained from Agricultural Research Center, Giza, Egypt. The animal groups were kept in an atmosphere of filtered, pathogen-free air, water, and a temperature of 20-25ºC for 8 weeks, with a 12-hour light/dark cycle and a light cycle (8-20 h) and a relative humidity of 50%. For one week, all rats were fed a basal diet. The basal diet was designed to contain 14% casein, 10% sucrose, 4% corn oil, 5% fiber (cellulose), 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 (Reeves *et al.,* 1993). All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals. The experiment was conducted at Agricultural Research Center, Giza, Egypt. The rats were weighed weekly through the experimental period.

After acclimatizing for seven days prior to the study. Rats were weighed, and randomly divided into six groups of six rats each. The first group of rats, the control (ve-) consists of (6) rats fed on standard diet for 8 weeks. The second group (injected rats) consists of (30) rats injected with Carbon tetrachlorideCCl$_4$ in paraffin oil (50%v/v) at the level of 2 ml/kg of body weight as carcinogens twice per week by subcutaneous injection for two weeks (Jayasekhar *et al*., 1997). They were then divided into five subgroups of six rats each and fed different proportions of spirulina powder added to processed cheese for 8 weeks divided as follows: control (ve +) group fed only the standard diet, processed cheese only group, processed cheese with spirulina powder (2%) group, processed cheese with spirulina powder (4%) group and processed cheese with spirulina powder (6%) group.

Biological Determination

Biological evaluation of the different tested diets was carried out by determination of food intake (FI), body weight gain% (BWG %) and organs weight/body weight% according to Chapman *et al.* (1959):

BWG% = [(Final weight - Initial weight) / (Initial weight)] X 100

Organ weight/ body weight % = (Organ weight / Final weight) X 100

Biochemical analysis

Blood samples were withdrawn from orbital plexus venous by using fine capillary glass tubes, placed in centrifuge tubes without anticoagulant and allowed to clot. After the serum prepared by centrifugation (3000 rpm for 15 min), serum samples were analyzed by bio diagnostic kits: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined calorimetrically using spectrophotometer (model DU 4700) at 505 nm according to the method of Reitman and Frankel (1957). Total protein was determined at 550 nm according to the method described by Gornal *et al*. (1949). Serum uric acid was determined after Barham and Trinder (1972) using spectrophotometer (model DU 4700) adjusted at 510 nm. Serum urea nitrogen was determined according to the method described by Batton and Crouch (1977) using spectrophotometer (model DU 4700) adjusted nm 550 nm. Serum creatinine was determined by Tietz (1986) using spectrophotometer (model DU 4700) adjusted at 510 nm. Determination of Serum cholesterol was determined according to the method described by Allain *et al*. (1974) using spectrophotometer (model DU 4700)

adjusted at 578 nm. Serum Triglycerides (TG) were determined according to the method described by Fossati and Principe (1982) using spectrophotometer (model DU 4700) adjusted at 500 nm. High-density lipoprotein cholesterol (HDL-$_c$) was determined according to the method described by Burstein, (1970) using spectrophotometer (model DU 4700) adjusted at 520 nm. Low-density lipoprotein cholesterol (LDL-$_c$) was determined according to the method described by Friedwald *et al*. (1972).

Histopathology Technique

The tissues sample from liver and kidney were fixed immediately after dissection in 10% neutral formalin for 24 h, then collected and dehydration was done on ascending concentrations of alcohol, cleaned in xylene and embedded in paraffin wax. Tissues were sectioned at a thickness of 3 micron and stained with hematoxylin and eosin stains (Banchroft *et al.,* 1996). And examined by the light microscope for detection of any histopathological alteration.

Statistical Analysis

The data obtained from the present study was statistically subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1980) by the computerized program SPSS software, version "20" for Windows. The least significant difference (LSD) value was used to determine significant difference between means. Data was represented as Mean ± SD. Values were considered significant at $P \leq 0.05$, otherwise were considered non-significant

RESULTS AND DISCUSSION

Data in Table (1) demonstrate the effects of CCl_4 induction and spirulina supplementation on body weight in experimental groups. All groups started with similar initial body weight (ranging from 198.66 ± 3.5 to 205.33 ± 1.51 g), indicating homogeneity among the groups at the beginning of the experiment. The control group (ve-) showed a final body weight of (269.33 ± 2.74 g), which was significantly higher than the CCl4-induced group (ve+) with (218.66 ± 2.51 g). This reduction in final body weight in the CCl_4-induced group is consistent with previous studies showing that CCl_4, a hepatotoxic agent, induces oxidative stress and liver damage, leading to impaired metabolism and weight loss (Al-Harbi *et al*., 2020; Abdel-Daim *et al*., 2019). Interestingly, the groups treated with processed cheese supplemented with spirulina showed a dose-dependent improvement in final body weight. The group treated with 6% spirulina achieved the highest final body weight (289.67 ± 1.96 g), even surpassing the control group. This suggests that spirulina, known for its antioxidant, anti-inflammatory, and hepatoprotective properties, may counteract the detrimental effects of CCl_4 and promote weight gain (Wu *et al*., 2016; Bashandy *et al*., 2021).

The control group exhibited a body weight gain % (33.29 ± 3.82%), while the CCl_4-induced group showed a significantly lower gain % (9.70 ± 1.12%), further confirming the negative impact of CCl_4 on growth and metabolism. However, spirulina supplementation at 2%, 4%, and 6% resulted in progressive improvements in weight gain, with the 6% spirulina group achieving the highest gain% (41.09 ± 2.83%). This aligns with studies indicating that spirulina enhances nutrient absorption, improves metabolic efficiency, and reduces oxidative stress, thereby promoting growth and weight gain (Khan *et al*., 2020; El-Sheekh *et al*., 2021).Spirulina's beneficial effects can be attributed to its rich composition of phycocyanins, carotenoids, and essential

amino acids, which have been shown to mitigate oxidative stress and inflammation induced by toxins like CCl_4 (Farag *et al*., 2022). Additionally, spirulina enhances liver function, which is critical for metabolism and nutrient utilization, thereby supporting weight gain (Bashandy *et al*., 2021). The combination of spirulina with processed cheese may also provide synergistic effects, as cheese is a source of high-quality proteins and fats that support growth and recovery. The current results are consistent with earlier research demonstrating the hepatoprotective and growth-promoting effects of spirulina. For instance, a study by Abdel-Daim *et al*. (2019) reported that spirulina supplementation significantly improved body weight and liver function in rats exposed to CCl_4. Similarly, Wu *et al*. (2016) found that spirulina enhanced growth performance and reduced oxidative stress in animal models. The dose-dependent effects observed in this study further validate the potential of spirulina as a therapeutic agent in mitigating toxin-induced weight loss and promoting recovery.

Table (1): Mean body weight gain (g) of experimental rats treated with processed cheese supplemented with (2,4 and 6 %) spirulina against carbon tetrachloride (CCl_4)-induced liver damage.

Body weight (g)	Groups					
	Control (ve-)	Inducted (ve +)	processed cheese only	treated by processed cheese +		
				2 % spirulina	4 % spirulina	6 % spirulina
IBW	202.00± 2.64 ^{b}	199.33± 2.08 ^{b}	201.66± 2.88 ^{b}	198.66±3.5^{b}	203.00±2.6a	205.33±1.51^{a}
FBW	269.33±2.74 ^{b}	218.66±2.51 ^{f}	245.33±1.4 ^{d}	242.66±2.5^{e}	266.00± 3.6^{c}	289.67±1.96^{a}
BWG %/wk	33.29±3.82^{b}	9.70±1.12 ^{e}	21.7100±2.71^{d}	22.17±2.14^{d}	31.04±0.55^{c}	41.09±2.83 ^{a}

Data are presented as means ± SD). Data in a row with different superscript letters are statistically different ($P \leq 0.05$).
IBW= Initial body weight; FBW= Final body weight; BWG=Body weight gain Wk = week

The results presented in Table (2) demonstrate significant variations in organ weight/body weight percentages across different experimental groups, particularly in the liver and kidney. The liver weight/body weight percentage was significantly higher in the CCl_4-induced group (5.52 ± 0.95%) compared to the control group (3.39 ± 0.83%). This increase is consistent with previous studies that have shown CCl_4-induced hepatotoxicity, leading to liver inflammation, fibrosis, and hypertrophy (Weber *et al*., 2003). The administration of processed cheese alone reduced the liver weight/body weight ratio to (4.24 ± 1.02%), indicating a partial protective effect. However, the most notable improvement was observed in groups treated with processed cheese supplemented with spirulina. The 2% spirulina group showed a liver weight/body weight ratio of (3.49 ± 0.4%), which is close to the control group, suggesting a significant hepatoprotective effect. This aligns with studies demonstrating spirulina's antioxidant and anti-inflammatory properties, which mitigate liver damage induced by toxins like CCl_4 (Wu *et al*., 2016). The 4% and 6% spirulina groups also showed reduced liver weights compared to the CCl_4-induced group, further supporting the dose-dependent protective role of spirulina.

Similarly, the kidney weight/body weight percentage was elevated in the CCl_4-induced group (0.89 ± 0.09%) compared to the control group (0.52 ± 0.09%). This increase is indicative of CCl_4-induced nephrotoxicity, which has been documented in previous studies (Ogeturk *et al*., 2005).

The administration of processed cheese alone reduced the kidney weight/body weight ratio to (0.73 ± 0.13%), while supplementation with spirulina further improved this metric. The 2% spirulina group showed a ratio of (0.65 ± 0.08%), and the 4% and 6% spirulina groups exhibited ratios of (0.78 ± 0.11% and 0.73 ± 0.04%, respectively). These results suggest that spirulina supplementation, particularly at lower concentrations, may help mitigate kidney damage induced by CCl_4. This is consistent with studies highlighting spirulina's nephroprotective effects, attributed to its antioxidant and anti-inflammatory properties (Moorhead *et al*., 2012). The 2% spirulina concentration appears to be the most effective in restoring organ weights to near-normal levels, suggesting its potential as a therapeutic agent in mitigating toxin-induced organ damage.

Table (2): Mean organ weight/body weight (%) of experimental rats treated with processed cheese supplemented with (2,4 and 6 %) spirulina against carbon tetrachloride (CCl_4)-induced liver damage.

Organ weight/body weight %	Groups					
	Control (ve-)	Inducted (ve +)	processed cheese only	treated by processed cheese +		
				2 % spirulina	4 % spirulina	6 % spirulina
Liver	3.39± 0.83^{d}	5.52± 0.95 ^{a}	4.24± 1.02 ^{b}	3.49±0.4^{c}	4.04±0.21b	3.96±0.58^{b}
Kidney	0.52±0.09 ^{e}	0.89±0.09 ^{a}	0.73±0.13^{c}	0.65±0.08^{d}	0.78± 0.11b	0.73±0.04^{c}

Data are presented as means ± SD. Data in a row with different superscript letters are statistically different ($P \leq 0.05$).

Biochemical Analyses

Liver Functions

Carbon tetrachloride (CCl_4) causes liver damage involving two phases; in the 1st phase, it is enzymatically converted by cytochrome P450 activity (CYP2E1) in hepatocytes to create trichloromethyl free radicals that are fatal metabolite (Sreedevi, 2005). It causes lipid peroxidation of the endoplasmic reticulum of the membranes of hepatocytes and initiates oxidative damage. The 2nd phase of hepatotoxicity involves the inflammatory reactions that perform a critical function. Certain hepatic cells including sinusoidal endothelial cells, Küpffer cells and hepatic stellate cells (HSCs) generate exude cytokines that referee liver fibrogenesis (De Paula *et al*., 2010). Both radicals (trichloromethyl and trichloromethyl peroxyl free radicals) are highly reactive and initiate complex cellular modifications that result in hepatotoxic damage, inflammation, cirrhosis, fibrosis, and hepatocellular carcinoma (HCC) (Scholten *et al*., 2015).

Table (3) presents the effects of CCl_4-induced liver damage and the subsequent treatment with fortified processed cheese containing varying percentages of spirulina (2%, 4%, and 6%) on liver function markers, including ALT (alanine aminotransferase), AST (aspartate aminotransferase), and total protein levels. The results are compared across five groups: control (healthy), CCl4-induced liver damage (untreated), processed cheese only, and CCl_4-induced liver damage treated with spirulina-fortified processed cheese at different concentrations.

ALT and AST are key enzymes released into the bloodstream during liver damage, and their elevated levels are indicative of hepatocyte injury. In the CCl_4-induced group (ve+), both ALT and AST levels were significantly elevated (109.53 ± 1.89 and 72.28±1.4 U/l, respectively)

compared to the ve-control group(60.63 ± 1.28 and 39.96 ± 0.63 U/l, respectively), but the total protein was significantly lower in ve+ control (3.47 ± 0.03 g/dl) than ve- one (6.60 ± 0.82 g/dl). This is consistent with the known mechanism of CCl_4, which generates free radicals, leading to lipid peroxidation and cell membrane damage (Ashgan *et al*., 2015). This elevation is consistent with the known hepatotoxic effects of CCl_4, which induces oxidative stress and lipid peroxidation, leading to hepatocellular injury (Weber *et al*., 2003). However, treatment with spirulina-fortified processed cheese at 2%, 4%, and 6% concentrations resulted in a dose-dependent reduction in ALT and AST levels. Notably, the 2% spirulina group showed the most significant improvement, with ALT levels (60.88 ± 0.46 U/l) nearly returning to the control levels and AST levels (38.37 ± 1.87 U/l) even lower than the control. Also, restored total protein levels to near-normal values (6.50 ± 0.44 g/dl), indicating improved liver synthetic function. This indicates that spirulina, a rich source of antioxidants, may mitigate oxidative stress and stabilize liver cell membranes, thereby preventing enzyme leakage (Bhattacharyya *et al*. 2006). Also, a dose-dependent hepatoprotective effect, likely due to Spirulina's high antioxidant content, including phycocyanin, which scavenges free radicals and reduces oxidative stress (Mohamed *et al*., 2020). This is consistent with recent studies demonstrating spirulina's hepatoprotective and antioxidant properties, attributed to its high content of phycocyanins, carotenoids, and other bioactive compounds (Wu *et al*., 2016; Abdel-Daim *et al*., 2020). Also, this aligns with findings by Ismail *et al*. (2009), who reported that spirulina enhances liver repair mechanisms by promoting the synthesis of essential proteins and reducing oxidative damage.

The current data indicated that spirulina, particularly at the group treated with 2% spirulina-fortified processed cheese demonstrated the most significant improvement in liver function markers (ALT, AST, and total protein levels) compared to the groups treated with higher concentrations (4% and 6% spirulina). This phenomenon can be explained by several factors, supported by El-Tantawy (2015) who reported that low-dose spirulina supplementation improved liver function markers more effectively than high doses, likely due to better nutrient absorption and utilization. Also, Zheng *et al*. (2018) found that low doses of spirulina effectively reduced inflammation in liver injury models by downregulating TNF-α and IL-6 levels, while higher doses did not show proportional benefits. Kapoor and Mehta (1998) suggested that while spirulina is generally safe, excessive consumption can lead to nutrient imbalances and reduced efficacy in liver protection.

A study by El-Baz *et al*. (2019) demonstrated that dairy products, including processed cheese, can mitigate oxidative stress and improve liver function in animal models. Similarly, the addition of functional ingredients like spirulina to dairy products has been shown to enhance their therapeutic potential. For example, a study by Abdel-Daim *et al*. (2020) reported that spirulina supplementation significantly reduced liver enzyme levels and improved antioxidant status in rats with drug-induced liver injury. Processed cheese fortified with spirulina provides a rich source of antioxidants such as phycocyanin, β-carotene, and vitamin E, which neutralize free radicals generated by CCl_4 and prevent lipid peroxidation (Romay *et al*., 2003).Also, bioactive peptides in processed cheese and spirulina modulate inflammatory pathways, reducing liver inflammation and damage (Farooq *et al*., 2015) and the processed cheese contains high-quality proteins and essential amino acids that support liver regeneration and protein synthesis (Ismail *et al*., 2009).

Table (3): Liver functions (U/I) of experimental rats treated with processed cheese supplemented with spirulina against carbon tetrachloride (CCl_4)-induced liver damage.

Liver function (U/l)	Groups					
	Control (ve-)	Inducted (ve +)	processed cheese only	treated by processed cheese +		
				2 % spirulina	4 % spirulina	6 % spirulina
ALT	60.63± 1.28 ^{e}	109.53± 1.89 ^{a}	93.48± 1.32 ^{b}	60.88±0.46 ^{e}	77.27^{c}±1.45	91.3±2.18^{b}
AST	39.96±0.63 ^{e}	72.28±1.4 ^{a}	53.95± 0.58 ^{b}	38.37±1.87 ^{f}	46.61^{c}± 0.88	45.53±1.00^{b}
T. Protein	6.60±0.82^{a}	3.47±0.03 ^{e}	3.60±0.14 ^{d}	6.50±0.44^{a}	3.83±0.39^{b}	3.70±0.14 ^{c}

Data are presented as means ± SDM ($n=6$). Data in a row with different superscript letters are statistically different ($P \leq 0.05$). AST: aspartate amino transferase; ALT; alanine amino transferase

Kidney functions

The results presented in Table (4) demonstrate the effects of processed cheese supplemented with different concentrations of spirulina (2%, 4%, and 6%) on urea, creatinine, and uric acid levels in rats induced with CCl_4. In the control group (ve-) urea levels were 33.33 ± 1.85 mg/dl, while in the CCl_4-induced group (ve+) urea levels significantly increased to 51.50 ± 2.12 mg/dl, indicating renal impairment. However, treatment with processed cheese supplemented with spirulina at 2%, 4%, and 6% concentrations resulted in a dose-dependent reduction in urea levels. The 6% spirulina group showed the most significant reduction, bringing urea levels close to those of the control group (34.50 ± 0.71 mg/dl). This suggests that spirulina has a nephroprotective effect, likely due to its antioxidant properties, which mitigate oxidative stress induced by CCl_4. Similar findings were reported by El-Sheekh *et al*. (2020) who demonstrated that spirulina supplementation reduced urea levels in rats with drug-induced nephrotoxicity.

Creatinine levels in the control group were 0.87 ± 0.04 mg/dl, while the CCl_4-induced group showed a marked increase to 2.96 ± 0.18 mg/dl, indicating severe renal dysfunction. Treatment with spirulina-enriched processed cheese significantly reduced creatinine levels, with the 6% spirulina group showing the most pronounced effect (0.89 ± 0.06 mg/dl). This reduction in creatinine levels suggests improved renal filtration and function. These results align with those of Khan *et al*. (2019) who found that spirulina supplementation ameliorated creatinine levels in rats with cisplatin-induced nephrotoxicity, attributing this effect to spirulina's ability to scavenge free radicals and reduce oxidative damage. Uric acid levels in the control group were 1.59 ± 0.30 mg/dl, while the CCl_4-induced group exhibited elevated levels of 3.26 ± 0.17 mg/dl, indicative of impaired renal excretion. Treatment with spirulina-enriched processed cheese led to a significant reduction in uric acid levels, with the 6% spirulina group showing levels comparable to the control group (1.70 ± 0.14 mg/dl). This suggests that spirulina enhances renal excretion of uric acid, possibly through its anti-inflammatory and antioxidant mechanisms. Farooq *et al*. (2021) reported similar findings, highlighting spirulina's role in reducing uric acid levels in hyperuricemia rats.

Spirulina scavenges free radicals and reactive oxygen species (ROS) generated by CCl_4, thereby reducing lipid peroxidation and oxidative stress. Phycocyanin, a major

component of spirulina, has been shown to inhibit the production of ROS and enhance the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). This helps protect renal cells from oxidative damage and preserves their function **(**Khan *et al*., 2019**).** Also, Spirulina improves glomerular filtration rate (GFR) and enhances the excretion of waste products such as urea, creatinine, and uric acid. This is achieved by repairing damaged renal tubules and restoring their reabsorption and secretion capacities. Studies have shown that spirulina supplementation significantly reduces serum levels of urea, creatinine, and uric acid in animal models of renal injury **(**El-Sheekh *et al*., 2020**).**

Processed cheese serves as an effective vehicle for delivering bioactive compounds, such as spirulina's phycocyanin, antioxidants, and essential nutrients, to the body. The fat and protein content in cheese may enhance the bioavailability of these compounds, ensuring their efficient absorption and utilization. This could explain the dose-dependent improvements in renal parameters observed in the groups treated with spirulina-supplemented cheese (Finamore *et al*., 2017; Mooranian *et al*., 2019). These findings are supported by recent studies highlighting the benefits of dairy products and spirulina in managing oxidative stress and renal dysfunction (Khan *et al*., 2020).

Table (4): Kidney function (mg/dl) of experimental rats treated with processed cheese supplemented with spirulina against carbon tetrachloride (CCl_4)-induced liver damage.

Parameters (mg/dl)	Groups					
	Control (ve-)	Inducted (ve +)	processed cheese only	treated by processed cheese +		
				2 % spirulina	4 % spirulina	6 % spirulina
Urea	33.33±1.85^{d}	51.50±2.12^{a}	47.50±2.12^{b}	32.50±2.12^{d}	34.50±2.12^{c}	34.50±0.71^{c}
Creatinine	0.87±0.04^{d}	2.96±0.18^{a}	1.23±0.08^{b}	0.94±0.02^{c}	0.91±0.01^{d}	0.89±0.06^{d}
Uric Acid	1.59±0.30^{de}	3.26±0.17^{a}	2.53±0.11^{b}	1.81±0.21^{c}	1.84±0.09^{c}	1.70±0.14^{d}

Data are presented as means ± SDM (n=6). Data in a row with different superscript letters are statistically different ($P \leq 0.05$).

Lipid Profile

It was obvious from the results presented in Table (5) that there was a significant variations in lipid profile parameters (TC, TG, HDL-c, and LDL-c) across different experimental groups, including control, CCl_4-induced, and groups treated with processed cheese supplemented with varying concentrations of spirulina (2%, 4%, and 6%).The CCl_4-induced group exhibited a marked increase in TC, TG and LDL-c levels (155.33 ± 1.37, 127.50 ± 1.44 and 76.00 ± 1.22 mg/dl, respectively) meanwhile, HDL-c levels were significantly reduced (17.93 ± 0.29 mg/dl) compared to the control group (93.67 ± 1.37, 84.00 ± 4.24 and 40.33 ± 0.07 mg/dl and 29.00 ± 4.77 mg/dl, respectively).Oxidative stress caused by CCl_4 inhibits the activity of key enzymes involved in lipid metabolism, such as lipoprotein lipase (LPL) and lecithin-cholesterol acyltransferase (LCAT). This inhibition results in elevated levels of TC and TG, as well as reduced HDL-c levels (Abdel-Daim *et al*., 2019). CCl_4 triggers an inflammatory response in the liver by activating pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and

interleukin-6 (IL-6). Chronic inflammation further disrupts lipid metabolism by altering the expression of genes involved in lipid synthesis and transport (Weber *et al*., 2003; Manna *et al*., 2010). Inflammation reduces the expression of peroxisome proliferator-activated receptor alpha (PPAR-α), a key regulator of fatty acid oxidation. This leads to the accumulation of TG and TC in the liver and bloodstream (Huang *et al*., 2018). Additionally, inflammation impairs HDL-c function by reducing the activity of LCAT, an enzyme essential for HDL maturation (Panahi *et al*., 2020).

However, treatment with spirulina-supplemented processed cheese significantly reduced TC, TG and LDL-c levels in a dose-dependent manner. The most notable reduction was observed in the 6% spirulina group (57.50 ± 0.71 mg/dl), This aligns with previous studies demonstrating spirulina's ability to reduce TC through its antioxidant and hypolipidemic effects (Deng & Chow, 2010; Torres-Durán *et al*., 2007).This reduction may be attributed to spirulina's ability to enhance lipid metabolism and inhibit lipogenesis, as supported by studies indicating spirulina's role in reducing TC, TG and LDL-c levels (Nagaoka *et al*., 2005). Spirulina's hypocholesterolemia effects can be attributed to its high content of bioactive compounds, such as phycocyanin, gamma-linolenic acid (GLA), and antioxidants. These compounds inhibit cholesterol absorption in the intestines and enhance its excretion. A recent study by Ku *et al*. (2020) demonstrated that spirulina supplementation significantly reduces TC levels by modulating the expression of genes involved in cholesterol metabolism, such as HMG-CoA reductase and LDL receptors. Additionally, spirulina's antioxidant properties help mitigate oxidative stress, which is a key contributor to dyslipidemia (Wu *et al*., 2016). Also, Panahi *et al*. (2020) demonstrated that spirulina supplementation enhances HDL-c levels by reducing oxidative stress and improving the activity of lecithin-cholesterol acyltransferase (LCAT), an enzyme critical for HDL maturation. Additionally, spirulina's rich content of polyphenols and phycocyanin has been shown to enhance reverse cholesterol transport, further contributing to increased HDL-c levels (Moura *et al*., 2021). In the present study, spirulina was incorporated into processed cheese, which may have enhanced its bioavailability and efficacy. The combination of spirulina with dairy products provides a synergistic effect, as the calcium and proteins in cheese may further improve lipid metabolism and reduce cholesterol absorption (Zhao *et al*., 2020).

Table (5): Lipid Profile (mg/dL) of experimental rats treated with processed cheese supplemented with spirulina against carbon tetrachloride (CCl4)-induced liver damage.

Parameters (mg/dl)	Groups					
	Control (ve-)	Inducted (ve +)	processed cheese only	treated by processed cheese +		
				2 % spirulina	4 % spirulina	6 % spirulina
TC	93.67±1.37 ^{e}	155.33±1.37^{a}	115.33±0.71^{b}	57.50±0.71^{f}	105.50±0.71^{d}	109.50±2.12^{c}
TG	84.00±4.24^{b}	127.50±1.44^{a}	113.00±1.41^{c}	75.00±2.83^{f}	100.33±1.41^{d}	119.00±1.41^{b}
HDL-$_{c}$	29.00±4.77^{a}	17.93±0.29^{e}	22.70±1.27^{d}	26.10±0.14^{c}	26.90±0.42^{c}	27.00±1.70^{b}
LDL-$_{c}$	40.33±0.07^{e}	76.00^{a}±1.22	46.90±3.25^{d}	26.00±0.57^{f}	58.40±1.92^{c}	61.40±0.57^{b}

Data are presented as means ± SD. Data in a row with different superscript letters are statistically different ($P \leq 0.05$). LDL-C: Low density lipoproteins cholesterol.; HDL-C: Serum high density lipoproteins cholesterol; TC: Serum total cholesterol; TG: Serum triglyceride

Histopathological examination of liver:

Microscopic examination of liver sections of rats from all groups induced by CCl_4 then treated with (2,4 and 6 %) spirulina-supplemented processed cheese indicated that liver in the control (ve-) group has no histopathological alteration and with normal histological structure of its central vein and surrounding hepatocytes in the parenchyma (Fig.1-A). In adverse, the portal area of rats from control (ve+) group showed dilatation in the portal veins with newly formed bile ducts and proliferation of fibroblasts (Fig.1-B).These findings are consistent with previous studies that have documented CCl_4-induced hepatotoxicity, characterized by inflammation, fibrosis, and fatty changes (Weber *et al*., 2003; Manibusan *et al*., 2007). The observed histopathological alterations are indicative of oxidative stress and inflammation, which are key mechanisms underlying CCl_4-induced liver injury (Recknag el *et al*., 1989).The hepatic capsule of processed cheese only group showed accumulation of fat cells, while the portal area had inflammatory cells infiltration, cystic dilatation in the bile ducts as well as accumulation of fat cells, suggesting that the cheese itself did not provide significant hepatoprotection. Meanwhile, liver of rats from (processed cheese + 2 % spirulina) group adverse no histopathological alteration (Fig.1-C). This suggests that spirulina, even at lower concentrations, possesses potent antioxidant and anti-inflammatory properties that can counteract CCl_4-induced damage. Spirulina's antioxidants effectively neutralize free radicals, reduce oxidative stress, and prevent lipid peroxidation, thereby protecting hepatocytes from damage (Bhat & Madyastha, 2001). These findings align with previous studies demonstrating spirulina's ability to scavenge free radicals and reduce oxidative stress (Wu *et al*., 2016). Otherwise, liver of rats from (processed cheese + 4 % spirulina) group detected dilatation in the central vein (Fig.1-D). On the other hand, the portal area of (processed cheese + 6% spirulina) group showed congestion in the portal veins with fibroblastic cells proliferation and fatty change was detected in individual hepatocytes. At higher spirulina concentrations (4% and 6%), the treatment effects were less pronounced (Fig.1-E & F). This dose-dependent variability may be attributed to the complex interplay between spirulina's bioactive compounds and the severity of CCl_4-induced damage. While spirulina is known to contain phycocyanin, carotenoids, and other antioxidants that protect against liver injury (Romay *et al*., 2003), the excessive intake of spirulina may overwhelm the liver's metabolic capacity, leading to an imbalance in lipid metabolism. This can result in the accumulation of triglycerides in hepatocytes, causing fatty changes (steatosis) (Romay *et al*., 2003).

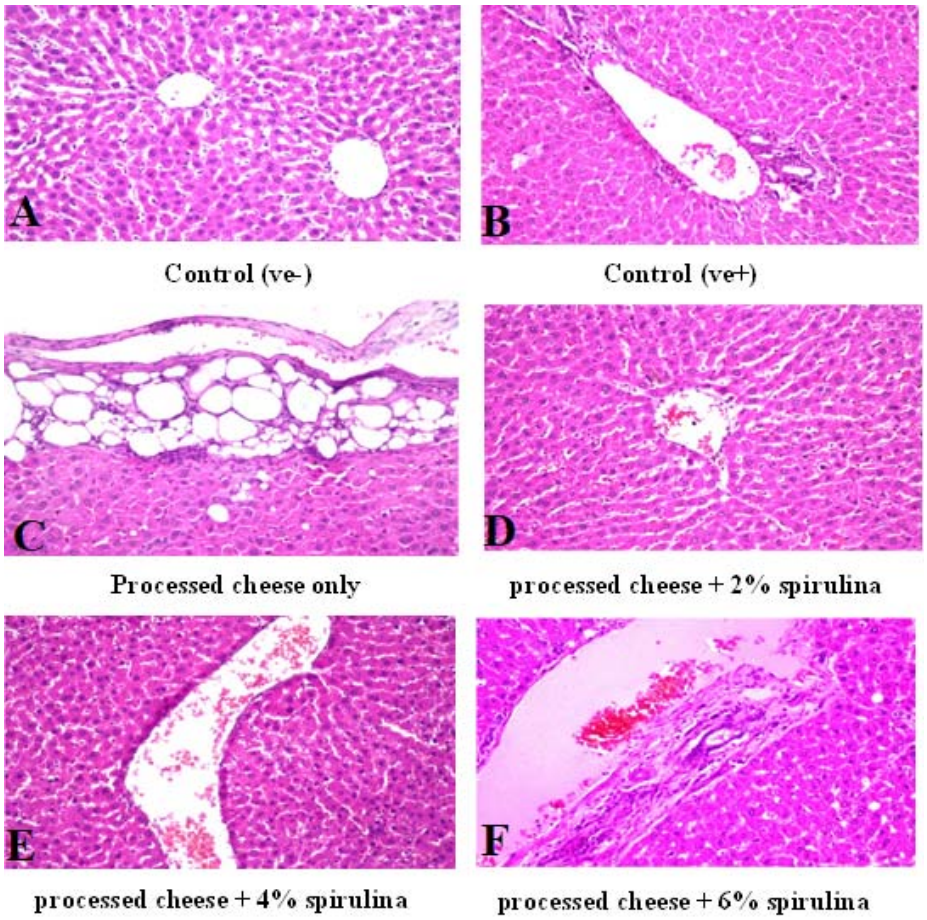

Fig. (1): Sections of liver in all experimental rats after treatment with (2,4 and 6 %) spirulina-supplemented processed cheese. Stained with hematoxylin – eosin. (X 200)

4.7.3. Histopathological examination of kidney:

Light microscopic examination of kidney sections of rats from all group's induced by CCl_4 then treated with (2,4 and 6 %) spirulina-supplemented processed cheese showed that kidney of the control (ve-) group has no histopathological alteration and with the normal histological structure of its glomeruli and tubules at the cortex (Fig. 2-A). On contrary, kidney of rats from control (ve+) group detected congestion and perivascular oedema in the cortical blood vessels (Fig. 2-B). kidney of rats from processed cheese only group described dilatation in the cortical blood vessels (Fig. 2-C). These findings are consistent with previous studies that have reported CCl_4-induced oxidative stress and inflammation as key contributors to renal tissue damage (Weber *et al*., 2003; Ogeturk *et al*., 2005). CCl_4 is known to generate free radicals, leading to lipid peroxidation and cellular injury in renal tissues (Manibusan *et al*., 2007). Makni *et al*. (2012) showed that Kidney histological sections in CCl_4-treated rats indicated glomerular hypertrophy and tubular dilatation.

On the other hand, kidney of rats treated with (2,4 and 6 %) spirulina-supplemented processed cheese showed exhibited apparent normal renal parenchyma

as seen in Figure (2- D, E & F) suggesting that spirulina mitigated the toxic effects of CCl_4. This protective effect can be attributed to the antioxidant properties of spirulina, which contains bioactive compounds such as phycocyanin, beta-carotene, and tocopherols, known to scavenge free radicals and reduce oxidative stress (Bhat & Madyastha, 2001; Romay *et al*., 2003). Also, these findings align with studies demonstrating the ability of spirulina to enhance cellular defense mechanisms and reduce oxidative damage in various tissues (Wu *et al*., 2016; Abdel-Daim *et al*., 2019).Spirulina's ability to preserve renal structure may be linked to its anti-inflammatory and antioxidant properties, which help maintain cellular integrity and function under conditions of oxidative stress (Khan *et al*., 2005; Gargouri *et al*., 2016). Furthermore, spirulina has been shown to modulate signaling pathways involved in inflammation and apoptosis, further contributing to its protective effects (Li *et al*., 2017).

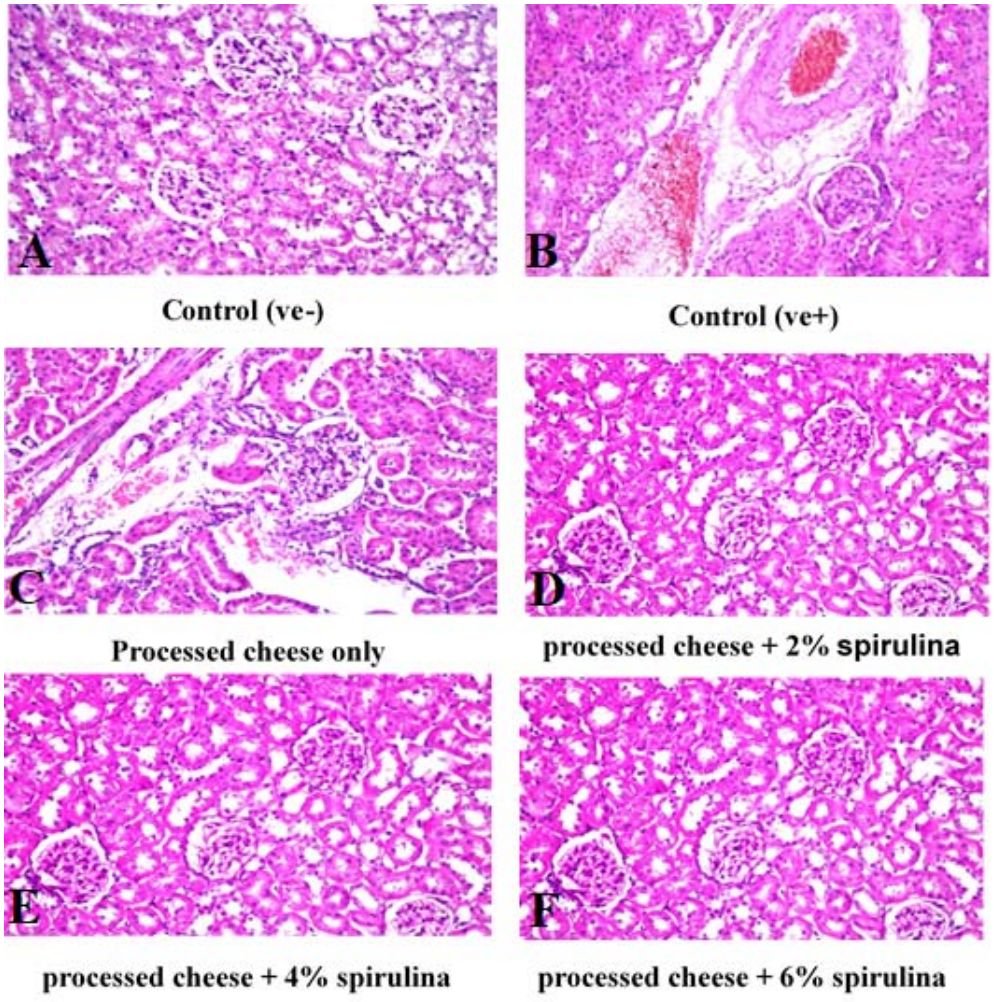

Fig. (3): Sections of kidney in all experimental rat after treatment with (2,4 and 6 %) spirulina-supplemented processed cheese. Stained with hematoxylin – eosin. (X200)

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تأثير الجبن المصنع إلمدعم بالاسبيرولينا على تلف الكبد و والسمية الكلوية لدى الفئران الناتج عن الاصابة برباعي كلوريد الكربون (CCl4)

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المستخلص

أُجريت هذه الدراسة لتقييم تأثير الجبن المطبوخ المُدعَّم بالسبيرولينا ضد تلف الكبد الناجم عن رابع كلوريد الكربون (CCl_4) في الفئران .تم وزن ستة وثلاثين من فئران ألألبينو وتقسيمها عشوائيًا إلى ست مجموعات تجريبية (كل مجموعة تحتوي على 6 فئران). تضمنت المجموعة الأولى (الضابطة السالبة {ve-}) 6 فئران تغذت على الغذائي القياسي لمدة 8 أسابيع. بينما تألفت المجموعة الثانية (الفئران المحقونة) من 30 فأر حُقنت برابع كلوريد الكربون (CCl_4) المذاب في زيت البارافين (50% حجم/حجم) بجرعة 2 مل/كجم من وزن الجسم مرتين أسبوعيًا عن طريق الحقن تحت الجلد لمدة أسبوعين، ثم قُسِّمت إلى خمس مجموعات فرعية (كل مجموعة 6 فئران) وتغذت على نسب مختلفة من مسحوق السبيرولينا المضافة إلى الجبن المطبوخ لمدة 8 أسابيع. شملت هذه المجموعات: مجموعة ضابطة موجبة (ve+)تغذت على الغذاء القياسى فقط. ومجموعة تتغذى على الجبن المطبوخ فقط, و مجموعة تتغذى على الجبن المطبوخ المدعم بمسحوق السبيرولينا (2%), ومجموعة الجبن المطبوخ المدعم بمسحوق السبيرولينا (4%), ومجموعة الجبن المطبوخ المدعم بمسحوق السبيرولينا (6%).

أظهرت النتائج انخفاضًا ملحوظًا ($p \leq 0.05$) في مستويات التحاليل الكيميائية الحيوية لوظائف الكبد والكلي ، مع انخفاض واضح في مستويات الدهون. كما أظهر الفحص النسيجي للكبد والكلى في الفئران المحقونة بـ CCl_4 والمغذاة على الجبن المطبوخ المدعّم بالسبيرولينا تحسنًا مقارنةً بالمجموعة الضابطة الموجبة (ve+).