Effect of kale, lupine and its mixture sprouts on the biochemical parameters of hepatotoxicity rats with CCl4

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Abstract

The liver is the primary organ responsible for the metabolism and elimination of foreign chemicals. Carbon tetrachloride (CCl4) can cause liver damage by converting into trichloromethyl radical [CCl3]- through the action of multiple cytochrome P450 isoforms. Subsequently, [CCl3]- undergoes a reaction with oxygen to produce the trichloromethyl peroxyl radical [CCl3OO]-, which induces hepatoprotective activity. The current study aimed to investigate the impact of kale, lupine, and their combination on the hepatotoxicity that CCl4 induces in male rats. Forty two male albino rats weighing (180± 5 g) were divided into seven groups: The first group (6 rats) was fed on basal diet and kept as a negative group. The other groups were treated by injection with 2 ml/kg of weight rat of CCL4 diluted with liquid paraffin (1:1 v: v) weekly during experiment period to induce hepatotoxicity. Group (2) was kept as a positive group . Groups (3&4) were given CCl4 with 5 and 10% lupine sprouts when Groups (5&6) were given CCl4 with 5 and 10% kale sprouts and the last group was given CCl4 with 10% mixture of both sprouts. Chemical composition and antioxidants activity were determined. Also, Body weight, feed intake, feed efficiency ratio, fasting blood glucose, insulin resistance, liver enzymes, kidney functions and oxidative stress parameters were estimated. Lupine had high protein, total flavonoids and ABTs %while kale had high in fat, fiber ,carbohydrates, total phenols and DPPH. The biochemical, MDA and CP data of positive control group showed elevated except HDL-c and other oxidative stress parameters. . The best results were recorded for the groups treated with CCl4 and were given10% of tested sprouts followed by 5%.lupine sprouts had high effect on the tested parameters followed by the mixture of both sprouts and the last was kale sprouts . So, it was concluded from the present results that administration of lupine, kale and its mixture can ameliorating CCl4 hepatotoxicity which might be due to their biologically active compounds and antioxidant.

Key words: kale; sprouts; biochemical parameters; CCl4; antioxidants stress

Introduction

In the right upper quadrant of the abdomen, beneath the diaphragm, is the location of the liver. The process of red blood cell decomposition, regulation of glycogen storage, and production of hormones are some of its additional metabolic functions. Only vertebrates possess the liver organ which produces essential biochemicals for digestion and growth, detoxifies various metabolites, and synthesizes proteins. The liver is the principal organ of the body and is responsible for the metabolism and detoxification of foreign substances that enter the body. Despite possessing a significant capacity for regeneration, the liver is accountable for the harm inflicted by chemicals, pharmaceuticals, air pollutants, and drugs. Liver diseases are widespread and the primary cause of mortality among humans, they progress from steatosis to chronic hepatitis, hepatocellular carcinoma, fibrosis and cirrhosis **(Gao *et al.*, 2008; Molina *et al.*, 2015**

and Domitrović *et al.*, 2015). Carbon tetrachloride (CCl4) is a chemical agent that is frequently utilized in animal experiments to induce liver fibrosis and fatty liver **(Grover *et al*., 2002).** CCl4 exerts its hepatotoxic effects through the generation of an excessive number of free radicals, including the trichloromethyl radical (CCl_3) and the trichloromethylperoxy radical ($OOCCl_3$), which are accompanied by additional free radicals such as O_2– and H_2O_2. As a result, oxidative stress is induced due to the depletion of reductants (e.g., glutathione, GSH) and the inhibition of antioxidant enzymes (e.g., catalase (CAT) and superoxide dismutase (SOD)). Upon binding to phospholipid molecules encapsulated within the membranes of mitochondria, hepatocytes and the endoplasmic reticulum, toxic metabolites and free radicals induce lipid peroxidation and membrane dysfunction or damage. Furthermore, they can form bonds with additional macromolecules, including DNA and proteins, which can cause cell damage or death. This condition has the potential to worsen hypoxia, stimulate lipid accumulation, facilitate bacterial translocation, and gut leakage, stimulate cytokine release, and improve hepatic iron accumulation—all of which contribute to the acceleration of highly reactive radical production (**Weber *et al*., 2003 and Nurrochmad *et al*., 2013).** In the realm of drug discovery, plants have also made important contributions to medicine. Certain drugs continue to be obtained through direct extraction or chemical transformation, whereas others are synthesized **(Tolstikova *et al.*, 2009).** The sprouts produced from the seeds of numerous grain crops are deemed safe for human consumption. Sprouts are among the most complete and nourishing foods, according to scientific research. Sprouts are classified as predigested foods due to their reduced levels of antiphysiologic factors and higher biological efficiency values in comparison to raw or cooked seeds. Sprouts have been found to greatly improve the immune system due to their excellent detoxifying properties (**Wieca *et al.*, 2015 and Abdallah, 2017).**

The flowering lupine (*Lupinus angustifolius*) is classified as a member of the leguminosae (*fabaceae*) family. It is indigenous to North and South America, the Mediterranean, and North Africa, and the lupinus genus is a cool-season legume plant ***(*Sipsas, 2008 and Griffiths *et al.*, 2021).** Widely utilized in the food industry, lupin seeds are an exceptional source of minerals, dietary fiber, protein, and fat, and they also contain an abundance of nutritional value. In addition, lupine protein isolates and concentrates are a source of nutrition due to their unsaturated fats, high protein content, and indigestible starches, as well as their excellent technofunctional qualities, which are physical properties such as foaming and emulsification, enable their use in a wide variety of food products, including meat substitutes and baked goods. It has been established that human consumption is safe. Unlike the other spicease, which contained trypsin inhibitors, alkaloids, lectins, saponins, phytic acid, and protease inhibitors—commonly identified as the primary agents responsible for flatulence—this one contains only trace amounts of potentially toxic and bitter alkaloids. Animal energy utilization is further hindered by non-starch polysaccharides, whereas alkaloids typically diminish palatability and feed intake due to their bitter flavor. Seed germination can alter the composition and amount of nutrients present, thereby potentially impacting the animal's digestibility of protein and amino acids **(Kim *et al.*, 2007** ;**Sangronis & Machado, 2007 ; Abraham *et al*., 2019 and Šćiban, et al., 2021)**. **Chilomer *et al.* (2012) and Taglieri *et al. (* 2021)** the sprouts of yellow seeds exhibited a higher protein content and lower concentrations of antinutritive factors in comparison to the raw seeds. By decreasing the concentration of alkaloids and oligosaccharides, germination of lupine seeds may enhance their palatability and utility.

Kale, scientifically known as *Brassica oleracea*, is a member of the Brassicaceae family, which additionally comprises brussel sprouts, cauliflower, broccoli, and arugula. Kale sprouts are one of the superfoods of the 'broccoli' family. They're an excellent source of vitamin C, folic acid, calcium and potassium. Just 100g of kale sprouts contains double the amount of both vitamin B6 and vitamin C compared to standard mature kale plants. Health benefits have been related to an excellent mixture of bioactive phytochemicals, which comprises carotenoids, glucosinolates, and phenolic compounds. Carotenoids, glucosinolates, and phenolic compounds derived from kale sprouts are secondary metabolites that are beneficial to health and exhibit a range of pharmacological effects that correlate to their antioxidant properties. Anticancer, antioxidant, and protective effects on the cardiovascular and gastrointestinal systems are the primary biological activities related to kale sprouts **(Šamec *et al.*, 2019 and Luang *et al.*, 2020) .**

So, the purpose of this study was to examine the biological and biochemical effect of lupine, kale sprouts, and their combination on rats exposed to CCl4-induced hepatotoxicity.

Material and methods

Materials

The *Lupinus angustifolius* (sweet lupine) and *Brassica oleracea var. acephala* (kale) seeds were obtained from Agriculture Research Center, Cairo, Egypt. Casein, starch , all vitamins, minerals, cellulose, L –Cystine, CCl4, choline chloride and biochemical Kits were obtained from El–Gomhoriya Company for Chemicals and Drugs, Giza, Egypt. In Cairo, Egypt, corn oil was obtained from the local market. The Laboratory Animal Colony provided 42 Sprague Dawley strain normal male albino rats. Ministry of Health and Population, Helwan, Egypt.

Methods

Sprouts preparation

Seeds of sweet lupine were washed with potable water through fine-mesh frame sieves. The purpose of this procedure was to eliminate saponins, and it was carried out until no further foaming formed. Soaking lupine and kale sprout in 2.5 g l−1 sodium hypochlorite for 30 minutes was performed in order to minimize the microbial degradation that occurs during the germination process. The seeds were then rinsed to a neutral pH with distilled water. Following this, the seeds were germinated for a duration of 12 days at a temperature of 24°C in the dark using distilled water in open containers that were ventilated every day and were watered twice. After 72 hours of drying in an air oven at 35°C, sprouts were ground in a laboratory mill. For the performance (**Schabes and Sigstad , 2004).**

Determination of chemical constitutes

The concentrations of fiber, fat, total protein, moisture and ash in triplicate samples of sprouts that were tested were determined in accordance with **(A.O.A.C, 2012)**, while the estimation of total carbohydrates was performed using deference's method.

Total flavonoid determination

Aluminum chloride colorimetric method described by **Meda *et al*. (2005).**

Total phenols determination

Utilizing the Folin Ciocalteu reagent and the methodology outlined by **Singleton and Rossi (1965)**, the phenolic content of each molina fruit was determined.

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH radical scavenging activities of peanut shell were determined using the methodology outlined by **Moire et al. (2001)**.

Determination of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid(ABTS)

The ABTS radical scavenging activity of tested sprouts was carried out following the procedure of **Re *et al.* (1999).**

Biological design

The basal diet consisted of 10 % corn oil, 10 % protein (casein), 5% fibers (cellulose), 1% vitamins mixture, 4% salt mixture, and 2% choline chloride and the remainder were corn starch till 100%. Rats of normal male albino From the Laboratory Animal Colony, Sprague Dawley Strain (42 rats) weighing (180±5 g) were obtained. As stated by **Reeves et al. (1993)**, the animals were housed in hygienic, well-ventilated cages at the Ministry of Health and Population, Helwan, Egypt, and were provided with a basal diet for a duration of one week in order to facilitate adaptation. In order to induce hepatotoxicity in accordance with **Li et al. (2013)**, 36 rats were injected weekly with a dilution of 1% v/v liquid paraffin used in CCL4 after the adaptation period, the remaining six rats remained without treatment. To ensure induction, the activities of the ALP, ALT and AST enzymes were assessed in the first and second main groups subsequent to injection. The rats were categorized into the following 7 groups: Group (1), which acted as a negative control, was fed a basal diet, group (2) were treated rats with CCl4 and fed on basal diet as a positive control group. While groups (3and 4) were treated rats with CCl4 and fed on basal diet with 5 and 10% lupine sprouts and groups (5 and 6) were treated with CCl4 and fed on basal diet with 5 and 10% kale sprouts whereas, group (7) were treated rats with CCl4 and fed on basal diet with 5%lupine sprouts and 5%kale sprouts.

Throughout the 28-day experimental period, dietary intake was documented daily, whereas body weights were assessed on a weekly basis. The body weight gain **(BWG) and** feed efficiency ratio **(FER)** were measured in accordance with **Chapman *et al*. (1959).**

Following an overnight fasting period, anesthesia, and sacrifice at the conclusion of the experiment, blood samples were obtained from the aorta of each individual rat. The blood samples were centrifuged at 3000 rpm for 10 minutes in a dry, sterile tube in order to separate the serum and determine biochemical parameters, which was stored in sterile, dry plastic tube at -20°C until analysis.

Triglycerides and total cholesterol were the biochemical parameters that were assessed in accordance with the method described by **Fossati and Prencipe (1982)** and **Allain (1974)** respectively, The determination of HDL, VLDL-c, and LDL-c was conducted using the methodologies outlined by **Lopez (1977)** and **Lee & Nieman (1996)**, respectively.

Glutathione s-transferases (GSTs), glutathione peroxidase (GPX), catalase (CAT) superoxide dismutase (SOD), total antioxidant capacity (TAC) and malondialdehyde (MDA) were measured by method of **Koracevic (2001); Zhao (2001); Diego (2011); Sun *et al*. (1988); Satoh (1978) and Ohkawa *et al*. (1979)** respectively. Aspartate aminotransaminase (AST) and alanine aminotransferase (ALT) were measured in accordance with **(Bergmeyer *et al*.,1978).**

The analyses for alkaline phosphatase (ALP) and total protein (Tp) were conducted in accordance with the respective methodologies outlined by **Roy (1970)** and **Okokon et al. (2013)**.

Albumin (Alb) and globulin (Glb) were obtained by **Fernandez *et al.*(1966)** and **Domas & Biggs(1971).** Uric acid, urea and creatinine were evaluated using the methods of **Baraham &Trinder(1972); Patton& Crouch(1977) and Henry(1974).** Creatine phosphokinase (CPK), monoaminooxidase (MAO) and acetylcholinestrse (AChE), were measured by the methods of **Rosano *et al.* (1976)**; **Mc Eween (1969) and Den Blaauwen *et al.* (1983)** respectively.
Fasting glucose (mmol/l), Serum insulin concentrations and The fasting glucose to insulin ratio were measured using the methods of **Silfen *et al*.(2001).** (HOMA-IR) index, which is calculated by dividing fasting plasma glucose (mmol/l) by fasting plasma insulin (mU/l).
As per the estimation made by **Hanley et al. (2002).**

Statistical analysis

Standard deviation and mean were utilized to present the results. One-way analysis of variance (ANOVA) was utilized to analyze the data for comparisons of multiple variables. Duncan's test was utilized as a post hoc test in accordance with the statistical package program for the comparison of significance between groups **(Armitage and Berry, 1987).**

Results and discussion

Proximate composition of lupine and kale sprouts is shown in Table (1). The highest protein and energy contents were found in the lupine sprouts (32.87 % and 355.71 k.cal), while kale sprouts had high contents of moisture, fat,ash, fiber and carbohydrates. There are no significant changes between the tested sprouts in moisture, carbohydrates, and energy content. Similar findings regarding protein content were documented by **MartínezVillaluenga et al. (2006)**, who discovered that lupine, following soybean and chickpea, ranked third in terms of protein quality and possesses a relatively favorable amino acid profile. Kale sprouts exhibited a protein content ranging from 20.97 to 24.37 g/100 g. Rainfall conditions, biochemical variety differentiation, and agrotechniques have an important impact on the total protein content. Average fat content of kale sprouts was 8.67 g/100 g, while another study reported that kale fat could potentially reach 10 g/100 g, accounting for 54% of the total fatty acid content **(Ayaz *et al.*, 2006) and Lisiewska *et al.*, 2008).**

Table (1): Proximate composition of lupine and kale sprouts

Variable	Moisture	Protein	Fat	Ash	Fiber	Carbohydrates	Energy
Lupine sprouts	7.65^{a} ±0.07	32.87 ^{a} ±2.03	6.79 ^{b}±1.32	3.66 ^{b}±0.01	8.25 ^{b}±1.33	40.78 ^{a}±6.83	355.71 ^{a}±2.91
Kale sprouts	8.04^{a}±0.43	24.04 ^{b}±5.12	9.03 ^{a} ±0.73	4.55 ^{a} ±0.11	11.03 ^{a} ±0.76	43.31 ^{a} ±8.19	350.67 ^{a}±9.76

Means in the same column with different letters are significantly different ($p \leq 0.05$) (n = 3)

The exploration of new dietary sources of phenolic compounds is a topic of important public health concern owing to their potential antioxidant activity. Although sprouts are recognized as an important source of these compounds, not all varieties of sprouts are created equal in terms of polyphenol content. There were statistically significant differences among the sprout different types examined in this study. (table 2): kale sprouts were the richest in total phenols and DPPH while lupine was high in total flavonoids and ABTS. DPPH and total phenols showed a positive correlation with the antioxidant activity of sprouts, with kale exhibiting the highest antioxidant activity, followed by lupine. **Khang et al. (2016)** demonstrated that the antioxidant activities of legumes increased significantly during germination. The sprouts are

regarded as essential constituents of a nutritious diet owing to their protein, enzyme, amino acid, vitamin, and mineral content. The sprouts exhibited higher DPPH and ABTS values following a 5-day growth period in comparison to the seeds. Sprouts exhibited notable change in phenolic composition and antioxidant activity; this may be attributed to the activation of endogenous enzymes and intricate biochemical metabolism (**Zhang *et al.*, 2020).**

Table (2): Total phenols, total flavonoids and antioxidants activates of lupine and kale sprouts

Variable	Total phenols µgGAE/g	Total flavonoid µgGAE/g	DPPH(%)	ABTS(%)
Lupine sprouts	145.32 ^{b} ±5.87	286.04 ^{a} ±11.56	41.11 ^{b} ± 9.54	87.46^{a}± 9.76
Kale sprouts	698.33 ^{a} ± 12.43	97.11 ^{b}± 8.04	70.22^{a} ± 7.76	48.44 ^{b} ± 3.87

Means in the same column with different letters are significantly different ($p \leq 0.05$) (n = 3)

After a treatment period of 28 days, there were differences in the mean body weights of the five experimental groups in comparison to the two control groups. The body weights of all sprout groups exhibited a significant increase when compared to the positive control group. Conversely, they exhibited a decrease when compared to the negative control group (Table 3). Following this, the weight gain observed in the 10% kale sprouts group was identical to that of the negative control group. Contrary to the negative control group, the weight gain rate in the remaining groups was significantly lower. The total food intake of the rats in the control group was significantly higher than that of the rats in each of the treatment groups, which is a critical observation. Specifically, the feed efficiency ratio and feed intake of the rats that were administered a high kale intake and a 10% sprout mixture were identical to those of the negative control group. In contrast, they consumed a significantly greater quantity of food than the other groups. In this investigation, feed efficiency is determined through the division of weight gain (g) by the weight of food consumed. The feed efficiency of the sixth and seventh groups was marginally greater than that of the control group, but not significantly higher than the other tested groups. Significantly greater feed efficiencies were observed in the sprouts group compared to the positive control group. The observed results align with the range of weight reductions documented in another study. Hence, the observed decrease in weight gain subsequent to CCl4 treatment seems to be attributable to a reduction in feed intake and an efficiency feed ratio (**Nurrochmad *et al.*, 2013)**. Sprouts are rich in enzyme content. This aids in digestion, stimulates chemical reactions and metabolism, and is an exceptional source of protein that, when treated with CCl4, can promote weight loss **(Zhang *et al.*, 2020).** kale sprouts were an excellent source of vitamin C, folic acid, calcium and potassium. Just 100g of kale sprouts contains double the amount of both vitamin B6 and vitamin C which can improve satiety and increase the feed intake and body weight **(Luang *et al.*, 2020).** Lupine seeds are an excellent source of unsaturated fats, high-protein content, and indigestible starches, all of which stimulate the metabolism in the gastrointestinal tract and promote weight gain **(Griffiths *et al.*, 2021)**.

Table (3): Body weight gain, feed intake and efficiency ratio of hepatotoxicity rats by affecting by lupine ,kale sprouts and its mixture

Variable	Negative control	Positive control	Rats fed on 5% lupine sprouts	Rats fed on 10% lupine sprouts	Rats fed on 5% kale sprouts	Rats fed on 10% kale sprouts	Rats fed on 5% lupine sprouts+5% kale sprouts
Body weigh gain (g)	42.74^{a}± 3.11	21.8^{e}± 0.87	27.54^{d}±2.03	35.56^{c}± 5.04	26.75^{d}±5.72	40.45^{a} ±7.91	39.68 ^{b}±2.05
Feed intake(g)	15.89^{a} ±2.76	10.56^{d}±2.96	11.99^{c} ± 2.94	13.95^{b} ± 0.76	11.57^{c}± 1.04	15.20 ^{a} ±0.78	14.99 ^{a} ±0.78
Feed efficiency ratio	0.097 ^{a}±0.011	0.74^{d}±0.021	0.82^{c}±0.013	0.091^{b}±0.003	0.083^{c}±0.004	0.095 ^{a} ±1.89	0.095 ^{a} ±1.89

Means in the same row with different litters are significantly different Significant (p≤0.05).

The effects of 5%, 10% kale, and 10% mixture sprouts on serum fasting glucose, insulin concentration, insulin sensitivity, and IR indices, as well as biochemical values, are detailed in Table (4). Significant reductions in fasting serum glucose, serum insulin concentration, and the IR index were observed in groups 4, 6, and 7, respectively, as the quantity of sprouts increased. Supplementation with 10% sprouts led to a reduction in serum insulin and the IR index, as measured by HOMA-IR, when treatment effects were compared between the tested sprout groups and the values of the two control groups. These findings align with the findings documented by **Šamec et al. (2019),** which demonstrated that kale sprouts can reduce plasma glucose and insulin levels in rats, in addition to increasing insulin sensitivity. It was also shown that flavonoids in kale sprout had hypoglycemic effect. Thus, there is the possibility that antioxidants (such as flavonoids, phenols and vit. C) and another component plausibly responsible is dietary fiber for the hypoglycemic effect and improved insulin sensitivity (**Nagata *et al.*, 2017 and Griffiths *et al.*, 2021**).

Lupine sprouts exhibited the most significant impact on the biochemical parameters. Hepatic glucose production (HGP) can be inhibited by insulin both directly and indirectly via its effects on adipose tissue, the pancreas, and the brain. Insulin stimulates glycogen storage of glucose by the liver. By stimulating glucose uptake in adipose and muscle tissue with insulin secreted by the liver, the starch content of these foods is reduced by 10% during the sprouting process, resulting in a reduced carbohydrate content. Moreover, they are an excellent source of fiber, which prevents blood sugar levels from spiking. There are two primary causes for incorporating sprouts into a diabetic meal plan (**Guemes-Vera *et al.*, 2021).**

Table (4): Fasting glucose, Insulin, Insulin sensitivity and HOMA-IR of hepatotoxicity rats by affecting by lupine ,kale sprouts and its mixture

Variable	Negative control	Positive control	Rats fed on 5% lupine sprouts	Rats fed on 10% lupine sprouts	Rats fed on 5% kale sprouts	Rats fed on 10% kale sprouts	Rats fed on 5% lupine sprouts+5 % kale sprouts
Fasting glucose(mmol/l)	7.07^{f}± 0.15	11.08^{a}± 0.05	10.54^{a}±0.74	8.82^{c}± 0.03	9.94^{b}±1.53	7.92 ^{e}±0.78	8.08^{d}±1.34
Insulin mU/l)	7.12 ^{a}± 0.875	3.93 ^{g}± 0.55	4.53 ^{f}± 0.007	5.95 ^{d}± 0.85	5.03 ^{e}± 0.07	6.07 ^{c}± 1.01	6.92 ^{b}± 0.87
Insulin sensitivity	2.89 ^{a}± 0.54	1.42 ^{g}± 0.46	1.76 ^{f}± 0.15	2.07 ^{d}± 0.45	1.88 ^{e}± 0.16	2.09 ^{c}± 0.99	2.11 ^{b}± 0.48
HOMA-IR	1.55 g± 0.04	2.99 a± 0.05	2.27 c± 0.44	2.08 f± 0.25	2.36 b± 0.94	2.19 e± 0.11	2.21 d± 0.04

Means in the same row with different litters are significantly different Significant (p≤0.05).

CCl4 injection considerably raised serum ALT, ALP, AST enzyme, T. Protein, albumin, and globulin levels in positive control rats as hepatotoxicity complications and oxidative stress compared to negative control rats (table 5). Administration of 5%, 10 of sprouts and 10% of its mixture enhanced the liver's function. A higher concentration of the sprouts under investigation demonstrated superior effects on liver functions compared to a lower concentration. Remarkably, the administration of the examined sprouts reduced the alterations in hepatic functions induced by the CCl4 injection, restoring them to levels comparable to those observed in GI. The ALT level attenuated by 91.55, 89.65 and 94.83 when the lupine, kale sprouts level 10 %and its mixture respectively, in a similar vein, ATS and ALP were promoted. However, albumin, T. Protein, and globulin levels in comparison to positive control group significantly enhanced when rats were administrated when 5 and 10% and their mixture. Rats treated with CCl4 exhibited a significant reduction in liver enzymes and liver functions. Rat liver enlargement caused by CCl4 insertion was due to fat accumulation within liver cells **(Nurrochmad *et al.*, 2013 and Ozdemi et al., 2022)**. Elevated concentrations of serum enzymes (AST, ALT) are indicative of cellular membrane damage and leakage within the liver due to CCl4 intoxication. The administration of sprouts resulted in a significant enhancement in the concentrations of liver enzymes (ALT, AST), these sprouts contain an abundance of flavonoids, including rutin, kaempferol, myricetin, quercetin, resveratrol, and Naringenin. The antioxidative and anti-inflammatory properties of these compounds have been demonstrated in rats with hepatic damage. Attenuated oxidative

stress complications were observed as a result of the increased content of polyphenols. By inhibiting TGF- β 1 and preventing the formation of liver fibrosis, sprouts may therefore provide enhanced liver protection **(Afonso *et al.*, 2020 and Luang *et al.*, 2020).**

Table (4): AST, ALT, ALP, albumin, Glupinee and total protein of hepatotoxicity rats by affecting by lupine ,kale sprouts and its mixture

Variable	Negative control	Positive control	Rats fed on 5% lupine sprouts	Rats fed on 10% lupine sprouts	Rats fed on 5% kale sprouts	Rats fed on 10% kale sprouts	Rats fed on 5% lupine sprouts+5% kale sprouts
AST	35.22^{d} ±6.72	100.74^{a} ±8.51	93.65^{b} ±4.89	80.01^{c} ±9.33	88.83^{b} ±10.42	73.03^{c} ±4.53	79.83^{c} ±8.35
ALT	37.59^{e} ±2.75	118.64^{a} ±4.27	107.39^{b} ±3.61	91.55^{c}±8.45	101.44^{b} ±8.43	89.65^{d} ±10.21	94.83^{d} ±6.61
ALP	88.93^{e} ±6.45	160.65^{a} ±10.23	145.45^{b} ±7.44	129.61^{c} ±5.29	132.05^{b} ±8.93	113.68^{d} ±9.43	119.73^{d} ±4.02
Total protein	58.79^{d} ±5.92	109.73^{a} ±7.04	97.07^{b} ±5.92	85.03^{c} ±3.01	92.52^{b} ±4.14	80.53^{c} ±7.54	84.93^{c} ±9.26
Albumin	32.02^{e} ±5.27	50.84^{a} ±6.02	47.62^{b} ±8.92	40.65^{c} ±3.91	45.77^{d} ±5.33	39.96^{b} ±8.07	42.33^{b} ±3.66
Glupinee	32.98^{d} ±7.82	63.94^{a} ±8.55	42.72^{b} ±9.03	39.93^{c} ±3.51	44.06^{b} ±6.06	35.95^{c} ±4.09	40.75^{c} ±1.79

Means in the same row with different litters are significantly different Significant ($p \leq 0.05$).

Table 5 indicates the findings of an investigation that assessed the nephroprotective properties of lupine, kale, and their combination at 5% or 10% concentrations against oxidative stress and hepatotoxicity induced by CCl4. Injection of CCl4 significantly increased the concentrations of serum urea, uric acid, and creatinine in G2 rats compared to kidney functions in GI. The administration of sprouts significantly lowered alterations in urea, creatinine, and uric

acid that were induced by CCl4 issues (**Ozdemi *et al.*, 2022)**. Evidently, the greatest improvement was observed when 10% sprouts were utilized, surpassing the effectiveness of 5% sprouts, in comparison to control rats (G1). Numerous experimental models have demonstrated a correlation between nephrotoxicity and oxidative stress; the outcomes of our study, which assessed kidney functions, corroborated this pattern with respect to organ function markers. The data presented clearly demonstrated the restoration of all renal functions when tested sprouts were administered for a duration of 28 days in dose-dependent manure. In G4, G6, and G7, the decreases in uric acid, creatinine, and urea were considerably greater than in the other treated groups. In rats that were injected with CCl4 and provided with the sprouts under investigation, kidney function parameters increased to their baseline levels. Rosemarinic acid, carnosic acid, caffeic acid and essential oil have been previously identified as the responsible components for the body's defense against free radical damage induced by oxidative stress, which is a positive effect of sprouts on the inhibition of kidney function (**Afonso *et al.*, 2020 and Luang *et al.*, 2020).**

Table (5): kidney function of hepatotoxicity rats by affecting by lupine ,kale sprouts and its mixture

Variable	Negative control	Positive control	Rats fed on 5% lupine sprouts	Rats fed on 10% lupine sprouts	Rats fed on 5% kale sprouts	Rats fed on 10% kale sprouts	Rats fed on 5% lupine sprouts+5 % kale sprouts
Creatinine	0.79^{e}±0.11	2.56^{a}±0.52	2.36^{b}±0.33	1.97^{d}±0.23	2.17^{c}±0.82	1.95^{d}±0.54	1.98^{d}±0.91
Urea	18.34^{d}±2.05	30.64^{a}±3.45	27.08^{b}±4.02	21.92^{c}±3.81	25.95^{b}±3.67	$20.43.05^{c}$±4.65	22.75^{c}±4, 52
Uric acid	1.23^{d}±0.68	3.87^{a}±0.76	3.15^{b}±0.74	2.16^{c}±0.99	3.23^{b}±0.77	2.01^{c}±0.061	2.11^{c}±0.42

Means in the same row with different litters are significantly different Significant (p≤0.05).

Table 6 presents the activity levels of CAT, GPX, SOD, CPK, MDA, AChE, and MAO, which collectively demonstrate that CCl4 induces oxidative liver damage. More precisely, CCl4 treatment significantly reduced the activities of CAT, GPX, SOD, TAC, MAO, AChE, and GSTs, whereas it increased the activities of MDA and CPK and control of these changes was

observed with treatments containing 5% and 10% sprouts, suggesting that in CCl4-induced hepatotoxicity, the sprouts under investigation can inhibit oxidative liver damage. The liver is safeguarded by natural compounds through the prevention of lipid peroxidation and the production of reactive oxygen species (ROS). Furthermore, as the product of lipid peroxidation, MDA indirectly indicates the generation of ROS within organisms; consequently, it induces oxidative stress and generates reactive radicals, which worsen cellular damage **(Ozdemi *et al.*, 2022).** In the present study, sprouts significantly increased the expression of SOD and GSH and dose-dependently decreased the activities of MDA and CPK, indicating that they can effectively inhibit oxidative liver damage induced by CCl4 **(Wang *et al*., 2018).**

Enhanced antioxidant activity has been demonstrated to result from the incorporation of phenolics, which is an intriguing observation given the increase in phenolics and antioxidants during the sprouting process. In vivo and in vitro, antioxidant properties are exhibited by biologically active components, including flavonoids and phenolic acids, which impede lipid oxidation chain reactions. Because of the presence of phenolic hydroxyl groups within polyphenols, phenolics are capable of scavenging and inhibiting free radicals. A number of phenolic acids inhibit hydrogen peroxide formation, superoxide anion, and hydroxyl radicals via the action of an effective antioxidant component. The current study confirms that sprout consumption may aid in the reduction of cellular oxidation (**Afonso *et al.*, 2020 and Luang *et al.*, 2020).**

Table (6): kidney function of hepatotoxicity rats by affecting by lupine ,kale sprouts and its mixture

Variable	Negative control	Positive control	Rats fed on 5% lupine sprouts	Rats fed on 10% lupine sprouts	Rats fed on 5% kale sprouts	Rats fed on 10% kale sprouts	Rats fed on 5% lupine sprouts+5% kale sprouts
CAT	78.54^{a}±3.02	39.43^{c}±6.72	41.79^{c} ±6.92	49.56^{b}±6.17	40.86^{c}±5.93	45.93^{b}±6.53	48.01^{b}±4.53
GPX	86.22^{a}±1.21	56.03^{d}±8.13	65.04^{c}±4.06	73.39^{b}±2.14	63.08^{c}±6.18	70.06^{b}±7.16	72.44^{b}±2.16
SOD	52.95^{a}±0.99	30.45^{d}±8.39	37.08^{c}±5.13	47.17^{b}±7.33	35.34^{c}±6.12	44.48^{b}±2.42	45.32^{b}±7.11
GSTs	30.43^{a}±0.84	15.26^{d}±0.31	21.43^{c}±1.02	27.33^{b}±0.73	20.84^{c}±1.22	26.04^{b}±0.73	26.93^{b}±3.61
MDA	11.07^{d}±2.	37.86^{a}±3.	32.08^{b}±4.14	26.08^{c}±4.	34.27^{b}±4.04	29.67^{c}±2.45	27.67^{c}±2.45

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TAC	1.33^{a}±0.33	0.73^{e}±0.16	0.97^{d}±0.04	1.22^{b}±0.05	0.90^{c}±0.11	1.24^{b}±0.21	1.21^{b}±0.21
MAO	22.63^{a} ± 0.56	9.04^{d} ± 1.22	14.87^{c} ± 1.77	18.21^{b} ± 2.04	13.94^{c} ± 1.85	17.99^{b} ± 1.76	18.09^{b} ± 0.99
AChE	6.28^{a} ± ±1.98	1.42^{d} ± 0.8	2.99^{c}± 0.16	4.79^{b} ± 0.0	2.01^{c}± 0.06	4.31^{b} ± 0.19	4.51^{b} ± 0.76
CPK)	23.27^{d} ± 4.84	36.36^{a} ± 2.72	34.09^{b} ±2.14	26.24^{c} ± 3.41	33.08^{b} ± 2.66	29.07^{c} ± 2.61	27.07^{c} ± 1.88

Means in the same row with different litters are significantly different Significant (p≤0.05).

The impact of lupine, kale sprouts, and a 10% mixture of these substances on the lipid profile of rats induced with CCl4 hepatotoxicity was presented in Table (7). Mean serum triglycerides, total cholesterol, and lipoprotein fractions were significantly ($P < 0.05$) higher in the positive control group than in the negative control group, with the exception of HDL-c, which decreased in value. In contrast, the treatment groups consisting of lupine, kale sprouts, and their mixture exhibited significantly reduced values ($P< 0.05$) in comparison to the positive control group, while their values were significantly higher than those of the negative control group. The sprout groups had significantly higher mean HDL cholesterol ($P < 0.05$) than the positive control group and lower than the negative control group. The mean TG contents were significantly ($P < 0.05$) affected by the mixture of both sprouts more than the others whereas lupine, kale and its sprouts mixture had lower TC, LDL-c and VLDL-c levels and higher HDL-c and the differences in obtained results were non-significant. induction of hepatotoxicity by injection by CCL4 led to a significant increase in TC, TAG, LDL-c and VLDL-c, and decreased the HDL-c levels **(Grover *et al.*, 2002)**,. Phytoestrogenic flavonoids, specifically biochanin A and formononetin, which are more abundant in germinated seeds, were identified as the cause of the beneficial effects. Multiple studies have demonstrated that sprout consumption can reduce cholesterol levels in individuals with diabetes or obesity and exhibited a reduction in triglycerides and "bad" LDL cholesterol while increasing "good" HDL cholesterol. Furthermore, according to **Lisiewska et al. (2008)**, the findings of the present investigation are consistent with their findings, which reported that rats that were provided with kale had reduced total cholesterol and triglyceride levels, in addition to significantly lower levels of TAG in the serum, however, this modification in lipid profile was ameliorated through the inclusion of lupin sprouts in the diet. Blood cholesterol levels can be reduced, and cholesterol absorption and biosynthesis can be inhibited by phytochemicals in addition to their phytochemical content, dietary fiber found in kale and lupine sprouts has the potential to influence blood total cholesterol levels. It is common knowledge that sprouts rich in dietary fiber and resistant to starch reduce total cholesterol levels. By inhibiting bile salt (BS) reabsorption from the small intestine, which results in an excess of BS excreted in the feces; decreasing glycemic response, which inhibits insulin stimulation of hepatic cholesterol synthesis; and physiological effects of fermentation products of SDF, primarily propionate, soluble dietary fiber reduces total cholesterol levels **[Šamec *et al.*, 2019 and Ganopoulos *et al.*, 2019).**

Table (7): Lipid profile of hepatotoxicity rats by affecting by lupine ,kale sprouts and its mixture

Variable	Negative control	Positive control	Rats fed on 5% lupine sprouts	Rats fed on 10% lupine sprouts	Rats fed on 5% kale sprouts	Rats fed on 10% kale sprouts	Rats fed on 5% lupine sprouts+5% kale sprouts
Triglycerides	90.41 ^{e}±2.15	155.3 ^{a} ±7.23	141.4 ^{b}±8.25	129.02 ^{c}±9.07	145.11 ^{b} ±4.30	131.53 ^{c}±3.02	122.85 ^{d}±5.17
Total cholesterol	99.68 ^{d}±4.82	127.02 ^{a} ±3.81	113.31 ^{b}±7.19	109.074^{c}±6.03	112.502 ^{b}±4.33	108.8 ^{c}±7.08	107.34 ^{c} ±2.09
HDL-c	57.34^{a} ±2.82	35.92^{d} ±4.24	41.92^{c} ±6.95	49.63^{b} ±4.89	39.44^{c} ±5.02	45.16^{b} ±2.24	47.44^{b} ±4.89
LDL-c	28.42^{e} ±4.58	60.04^{a} ±9.82	43.11^{b} ±3.32	33.64^{d} ±7.08	44.04^{b} ±5.93	37.33^{c} ±9.51	35.33^{c} ±0.99
VLDL-c	18.082^{c} ±1.95	31.06^{a} ±2.05	28.28^{a} ±1.03	25.804^{b} ±2.53	29.022^{a} ±4.75	26.31^{b} ±2.68	24.57^{b} ±5.11

Means in the same row with different litters are significantly different Significant (p≤0.05).

Conclusion

Nowadays, the tendency to the study of the therapeutic properties of kale and lupine sprouts has expanded according to the available evidence. Kale and lupine have a high potential in the treatment of side effect of hepatotoxicity by CCl4 due to their active ingredients. Dietary intake of tested sprouts at 10% for 28 days may be beneficial for CCl4 hepatotoxicity by lowering higher level of serum liver enzymes, triglycerides and total cholesterol, enhances lipid fractions, glucose level and improve the antioxidants activities .

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