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Effect of Some Levels from Ziziphus Spina-christi Leaves and Choline on Acute Liver Disease in Rats

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Abstract

The study aims to investigate the effects of diets containing two levels of Ziziphus Spina-christi leaves, choline, and their combinationson nutritional evaluation, some biochemical analysis, anda histopathological examination of the livers of rats suffering from acute liver disease. This study involved the use of forty-eight male albino rats. Rats in this study were divided into2 main groups, as follows:The 1st main group (n=6 rats) was fed a basal dietas a negative control group. The 2ndmain group (42 rats) wasinjected subcutaneously with CCl4 in paraffin oil to induce acute liver disease in rats. The second main group of rats was divided into seven subgroups, each consisting of six rats,as follows: Subgroup (1)was only fed a basal diet (B.D.) as a control positive group. Subgroups 2 and 3were given diets containing 1% and 2% choline chloride, respectively. Subgroups 4 and 5were given diets containing 5% and 7.5% Zizyphus Spina-christi, respectively.Subgroups 6 and 7 were fed diets containing the combination of choline chloride and Zizyphus Spina-christi (1% choline chloride and 5% Zizyphus Spina-christi) and (2% choline chloride and 7.5% Zizyphus Spina-christi), respectively. The results indicated that injected rats with CCl4 increased liver enzymes, including AST, ALT, and ALP, while decreasing feed intake, body weight gain%, serum protein, albumin, globulin, and antioxidant enzymes, including (catalase "CAT", superoxide dismutase ("SOD"), glutathione ("GSH"), and glutathione peroxidase ("GPx"), as compared to non-injected rats. Treating acute liver disease rats with diets containing the two levels of Ziziphus Spina-christi leaves, choline, and their combinations improved all of these parameters and the histopathological changes in the liver as compared to non-treated rats. Conclusion: Ziziphus Spina-christi leaves, choline, and their combinationscan be used to reduce the side effects of acute liver diseases.

Keywords: *Acute liver disease, Ziziphus Spina-christi leaves, Choline, Rats and Biochemical analysis.*

1. Introduction

Acute liver failure ALD causes abnormal blood tests without chronic liver disease, leading to coagulopathy and hepatic encephalopathy; nutrition's role in prevention and treatment is unclear **(Abenavoli et al., 2022).** Liver dysfunction patients face multifactorial nutritional issues, making malnutrition treatment challenging. Rapid nutritional assessment is crucial for improved quality of life and prevents complications **(Mandato et al., 2018)**. Acute liver disease (ALD) can lead to severe complications like bleeding and brain pressure, indicating severe damage to the liver and affecting 80–90% of its cells **(O'Grady, 2005).**

*Ziziphus Spina-christi, also known as Christ's Thorn Jujube, is a native plant with antibacterial, antifungal, antioxidant, anti-hyperglycemic, and pain-relieving properties, making it a popular medicine and food **(Jinous and Elaheh, 2012).** Ziziphus Spina-christi fruit is a rich source of essential dietary components and high in vitamin C, with a significantly larger amount compared to strawberries, oranges, and grapes **(Hamza et al., 2015)**.*

Sidr (Ziziphus Spina-christi) is a multifunctional tree with potential therapeutic effects against liver and urinary diseases, digestive issues, and other health issues due to the bioactive components found in its leaves and fruits, including antioxidants and polysaccharides ***(Bencheikh et al., 2021 and Cadi, 2020).*** *Ziziphus genus members contain saponins, tannins, flavonoids, cyclopeptide alkaloids, and phenolic compounds, which are used to treat chronic inflammatory diseases like atherosclerosis, obesity, diabetes, cancer, constipation, and neurological disorders* ***(Abdulrhman and Shadma, 2021)***.

Choline is essential for neurodevelopment and is oxidized to betaine, which is an important source of one-carbon units during folate deficiency.Choline is a crucial nutrient that plays a vital role in various processes, including lipid metabolism, and is essential for cells, mitochondrial membranes, andthe neurotransmitter acetylcholine **(Zeisel, 2006)**.Choline metabolism is primarily carried out in the liver, where it is found in phosphatidylcholine and other choline-containing phospholipids **(Zeisel and da Costa, 2009).**Choline is essential for human health, as it prevents fatty liver and muscle damage **(Da Costaetal., 2006).** Nutrition solutions deficient in choline led to development of fatty liver and liver damage in patients (**Buchman, 2001)**.

Therefore, the study aimed to evaluate the impact of certain levels of *Ziziphus Spina-christi* leaves and choline on rats suffering from acute liver disease.

2. Materials And Methods:

Materials:

The essential components of the diet, including casein, cellulose, vitamins, and minerals, also CCl4 obtained from El Gomhoriya Company, Cairo, Egypt. Starch and corn oil were procured from the local market. *Zizyphus Spina-christi* was obtained from the national market of Agricultural Herbs and Medicinal plants in Cairo, Egypt.

Rats: Forty-eight male albino rats of the Sprague Dawley strain, weighing 150 ± 10g, were obtained from the laboratory of the animal colony, Ministry of Health and Population, Helwan, Cairo, Egypt.

Kits: for biochemical analysis were purchased from Gamma Trade Company for Pharmaceuticals and Chemicals, Dokki, Egypt.

Methods:

Biological Part:

According to **Reeves et al. (1993)**, The study involved 48 male albino rats, weighing 150 ± 10g, kept in stainless steel cages and fed one week on a basal diet for adaptation in the biological studies lab. Faculty of Home Economics, Helwan University.

Rats were divided into two groups after 7 days of adaptation to a basal diet, and the second group was treated with CCl4 to induce liver damage. The first main group (n = 6 rats) was fed on a basal diet as a control negative group, and the second main group (n = 42 rats) was treated with CCl4 in paraffin oil (50% v/v, 4 ml/kg) by subcutaneous injection to induce acute liver disease in rats **(Jayasekhar et al., 1997).** After confirming the induction of the disease by determining AST, ALP, and ALP in the first and second main groups, the second main group was divided into seven subgroups, each consisting of six rats, as follows: **Subgroup (1):** fed on a basal diet (BD) only as a control positive group (+ve). **Subgroups 2 and 3** were fed diets containing 1% and 2% choline chloride, respectively. **Subgroups 4 and 5** were fed diets containing 5% and 7.5% *Zizyphus Spina-christi*, respectively. **Subgroups 6 and 7** were fed diets containing the combination of choline chloride and *Zizyphus Spina-christi* (1% choline chloride and 5% *Zizyphus Spina-christi)* and 2% choline chloride and 7.5% *Zizyphus Spina-christi*, respectively. Daily feed intake and body weight gain percent were assessed for the biological effects of different levels of choline chloride, *Zizyphus Spina-christi*, and mixtures during 28-day experimental period.

Rats were fasted overnight at the end of the experimental period, followed by anesthesia and sacrifice. Blood samples were collected from the aorta of each rat. To evaluate some biochemical parameters, serum was separated from blood samples using a centrifuge. These parameters, includingaspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) according to **Henry (1974), alkaline phosphatase** (ALP) **(Belfield and Goldberg 1971),** serum protein **(Gomal et al., 1949),** serum albumin **(Doumas and Biggs 1971)**, and the antioxidant enzymes, including catalase (CAT), superoxide dismutase dismutase (SOD), glutathione (GSH) according to **(Aebi, 1984; Beauchamp and Fridovich1971, Paglia & Valentine 1967, and Sushil et al., 1989)**, respectively.

Histopathological examination of liver: Liver tissues were collected post-sacrifice, fixed in formalin solution (10%), trimmed, washed, dehydrated, imbedded in paraffin, cut into 46-micron sections, and stained with haematoxylin and eosin stain according to **Sheehan and Hrapchak (1980).**

The obtained results including "nutritional and biological results" of each group were statistically analysed by using the SPSS package according to **Sendecor and Cochran (1979)**.

3. Results and Discussion:

Effect of Some Levels from *Zizyphus Spina-christi* Leaves and Choline on Feed Intake and Body Weight Gain% of Rats Suffering from Acute Liver Disease

Table (1): presents the effects of diets containing choline, *Zizyphus Spina-christi* leaves, and their combinations on feed consumption (g/day/ rat) and body weight gain % in rats with acute liver disease.

The study found that healthy rats consumed 19.166 g/ rat/day of a basal diet, while rats suffering from acute liver disease consumed 17.266 g/each rat/day. Feed intake in the positive control group was significantly lower than that in the negative control group. The table data indicates that all tested groups showed no significant differences in feed intake compared to the negative control group. The treatments demonstrated a significant increase ($p \leq 0.05$) in this parameter compared to the positive control group.

Table (1): Effect of Some Levels from *Zizyphus Spina-christi* Leaves and Choline on Feed Intake and Body Weight Gain% of Rats Suffering from Acute Liver Disease.

Parameters Groups		**Feed Intake g/day/each rat**	**Body Wight Gain %**
Healthy rats fed on basal diet (control - ve)		19.166 [a] ± 0.288	20.840 [a] ± 0.640
Acute liver disease in rats fed on a	basal diet (control + ve)	17.266[b] ± 0.680	13.850 [e] ± 0.217
	diet containing 1% choline	19.166 [a] ± 0.288	16.500[c] ± 0.300
	diet containing 2% choline	18.986 [a] ± 0.450	18.766[b] ± 0.321
	diet containing 5% ZS-C leaves	18.796 [a] ± 0.270	14.200 [d e] ± 0.200
	diet containing 7.5% ZS-C Leaves	18.833 [a] ± 0.152	14.466 [d] ± 0.151
	diet containing 1% choline and 5% ZS-C leaves	19.00 [a] ± 0.556	14.433 [d] ± 0.208
	diet containing 2% choline and 7.5% ZS-C leaves	19.200 [a] ± 0.200	14.600[d] ± 0.200

ZS-C: Zizyphus Spina-christi leaves

Mean values in each column with same letters are not significantly different.

The mean value of body weight gain percentage (BWG%) in acute liver disease rats (control positive group) fed on the basal diet showed a significant decrease $p \leq 0.05$ as compared to the negative control group fed on a basal diet (13.850 ± 0.217 vs. 20.840 ± 0.640%). Body weight gain % in the positive control group decreased by about 33.54% compared to the negative control group. CCl4 induced a serious decline in weight gain in rats when compared with the control group ($p = 0.0001$) **(Elhattab et al., 2022)**.

All groups which were treated with two levels from choline, ZS-C leaves, and their combinations showed a significant increase $p \leq 0.05$ in the mean value of BWG%, as compared to the positive control group. The study found that diets containing 1% and 2% choline significantly increased the BWG% of acute liver disease rats compared to diets containing 5% and 10% ZS-C leaves.The acute liver disease groups treated with diets containing ZS-C leaves and a combination of "choline and ZS-C leaves" showed no significant changes in BWG% between them.

Jason and David (1997) found that feeding choline, soybean meal, canola meal, or peanut meal led to linear weight gain, while **Ronald and Paul (2000)** reported that with increased dietary choline consumption increasing weight gain and diet consumption **Hamad et al., (2022)** found that normal rats

fed a diet supplemented with Ziziphus Spina-christi leaves had significantly lower body weight and fat mass than rats that were not fed the supplement.

Effect of Some Levels of *Zizyphus Spina-christi* Leaves and Choline on Liver Enzymes in Rats Suffering from Acute Liver Disease.

The effects of Zizyphus Spina-christi leaves, choline, and their combination on liver enzymes in rats with acute liver disease, as illustrated in Table (2).

The study found that injecting rats by CCl4 to induce acute liver disease significantly raised serum Aspartate aminotransferase AST enzyme, the positive control group experienced a 96.15% increase of AST in comparison to the negative control group. Treating acute liver disease groups with *Zizyphus Spina-christi* leaves, choline, and their mixture significantly reduced the mean value of serum AST enzyme as compared to acute liver disease group.The results reveals also no significant differences in serum AST enzyme mean values between all treated groups, except for the acute liver disease group treated with 1% choline.The mean values of serum AST enzyme in acute liver disease groups that were treated with a diet containing (2% choline, 5% ZS-C leaves, 7.5 % ZS-C leaves, and the combination between choline and ZS-C leaves) showed the best results in serum AST enzyme due to non-significant changes in these parameter compared to the negative control group.

The results in this table revealed a significant elevation ($p \leq 0.05$) in serum ALT enzyme levels among rats that were injected with CCl4 in comparison to the levels observed in healthy rats, with a 148.82% increase in the positive control group in comparison to the negative control group.Treatment of rats with acute liver disease with Zizyphus Spina-christi leaves, choline, and their combination at two different levels resulted in a notable reduction in the mean values of serum ALT enzyme levels compared to the positive control group.The data in this table show no significant changes in serum ALT enzyme values between treated groups, except for the acute liver disease group treated with 1% choline, which showed a significant increase.

The positive control group, injected with CCl4, showed a significant increase in serum ALP, with a 28.36% increase compared to the negative control group. The study found that treatment groups with *Zizyphus Spina-christi* leave, choline, and their combination significantly decreased serum ALP levels in comparison to the positive control group.The findings presented in the table indicate anon-significant changes in the mean value of serum ALP between all treated groups except the group of rats suffering from acute liver disease and treated with 5% ZS-C leaves.

In this respect, **Khattab (2012)** documented that CCl4 induced acute liver injury in rats. This was substantiated by a marked rise in the serum enzyme activities of aspartate and alanine aminotransferase (ALT & AST), alkaline phosphatase (ALP), and hepatic malondialdehyde (MDA), with a significant reduction in weight gain percent.

The effects of *Zizyphus Spina*-christi L. (ZSC) water extracts on CCl4-induced hepatic fibrosis were investigated by **(Amin and Mahmoud-Ghoneim in 2009)**.they reported that, the daily consumption of ZSC extract over an 8-week period significantly prevented and improved hepatic fibrosis. It also improved liver condition, as evidenced by decreased activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), restored malondialdehyde levels, and maintained antioxidant balance.

Sidr Fermented Fruit SFP-supplemented camel milk showed a significant reduction in plasma AST and ALT levels compared to the streptozotocin-injected control group **(El Sayed et al., 2022).**

The hepatoprotective benefits of SFP are attributed to its elevated phenolic acid and flavonoid concentrations, which have antioxidant properties capable of scavenging free radicals **(Khouchlaa et al., 2017).** These results agreed with the findings of **Bencheikh et al. (2019)** and **Dikhanbayeva et al. (2021)**, who observed hepatoprotective effects in diabetic rats through the use of Sidr fruit extract and fermented camel milk, resulting in improved liver function.

A diet deficient in methionine and choline (MCD) can lead to steatohepatitis in mice **(Mu et al., 2010).** In this respect, the study by **Hiroko et al (2013)** found that mice fed a methionine-choline-deficient diet (MCD) lost both body and liver weight after 30 weeks**. Anstee and Goldin., (2006) and Tsujimoto et al., (2008)** reported that choline deficiency can cause steatohepatitis, hepatic inflammation, and fibrosis in mice fed a diet deficient in both choline and methionine.

Low choline diets can cause liver damage due to abnormal phospholipid synthesis, lipoprotein secretion, mitochondrial dysfunction, and endoplasmic reticulum stress, affecting liver function based on genotype and estrogen status **(Karen and Steven., 2012)**.

Table (2): Effect of Some Levels of*Zizyphus Spina-christi* Leaves and Choline on Liver Enzymes of Rats Suffering from Acute Liver Disease.

Parameters / Groups		Liver Enzymes (U/l)		
		AST	**ALT**	**ALP**
Healthy rats fed on basal diet (control - ve)		39.00 ^{d} ± 2.00	28.00 ^{d} ± 3.00	578.33^{d} ± 5.65
Acute liver disease in rats fed on a	basal diet (control + ve)	76.50 ^{a} ± 4.50	69.67 ^{a} ± 6.11	742.33 ^{a} ± 11.06
	diet containing 1% choline	57.67^{b} ± 3.51	52.00^{b} ± 6.00	668.33 ^{c} ± 6.35
	diet containing 2% choline	48.00$^{c\ d}$ ± 2.00	41.00^{c} ± 2.65	653.33 ^{c} ± 10.50
	diet containing 5% ZS-C leaves	43.67 $^{c\ d}$ ± 1.53	35.00 ^{c} ± 3.00	703.33^{b} ± 5.77
	diet containing 7.5% ZS-C Leaves	43.66 ^{cd} ± 3.79	35.00 ^{c} ± 1.00	660.67 ^{c} ± 8.08
	diet containing 1% choline and 5% ZS-C leaves	44.00 $^{c\ d}$ ± 3.00	40.00 ^{c} ± 3.00	659.33 ^{c} ± 11.93
	diet containing 2% choline and 7.5% ZS-C leaves	44.00 $^{c\ d}$ ± 2.65	41.00 ^{c} ± 1.00	671.33 ^{c} ± 14.74

ZS-C: Zizyphus Spina-christi leaves

Mean values in each column with same letters are not significantly different.

Betaine supplementation enhances liver oxyradical scavenging activity, reduces alcohol-induced serum ALT and AST elevations, and prevents blood-alcohol cycle, thereby reducing alcohol levels **(Li et al., 2011; Jung et al., 2013 and Yang et al., 2017)**. Betaine is a substance that protects against the risk of acute liver failure and toxin-induced liver damage **(Junnila et al.,1998)**. Reduced toxic effects of CCl4 on cell organelles **(Junnila et al.,2000).** Additionally, betaine administration reduced CCl4-induced fibrosis by preventing lipid peroxidation, liver inflammation, and transforming growth factor-β1expression **(Tsai et al.,2015).** Betaine supplementation restores glutathione content and antioxidant enzyme activities in mice with metabolism-associated fatty liver disease MAFLD.

Effect of Some Levels from *Zizyphus Spina-christi* Leaves and Choline on Protein Status in Rats Suffering from Acute Liver Disease.

Injecting rats fed on a basal diet with CCl4 to induce acute liver diseases decreased the mean value of protein status, including serum (protein, albumin, and globulin), as compared to non-injected rats fed on the same diet (Table 3).

Treatment of acute liver disease groups with two levels of choline, *Zizyphus Spina-christi* leaves, and their combination led to a significant increase ($p \leq 0.05$) in serum protein in all treated groups, except for the group of rats treated with a diet containing 1% choline as compared to the positive control group. Diet containing 2% choline and 7.5% ZS-C leaves recorded the best results in serum protein. Treatment of acute liver disease groups with choline, *Zizyphus Spina-christi*leaves, and their combination showed non-significant differences in serum albumin as compared to the positive control group. On the other hand, all treated groups that were suffering from acute liver disease with tested diets recorded significant increases in serum globulin, except for groups of rats treated with diets containing "1% choline" and "5% ZS-C leaves", as compared to the positive control group. The best results in protein status, including serum protein, albumin, and globulin, were recorded for the acute liver disease group treated with high levels of the combination of 2% choline and 7.5% *Zizyphus Spina-christi* leaves.

Table (3): Effect of Some Levels from *Zizyphus Spina-christi* Leaves and Choline on Protein Status in Rats Suffering from Acute Liver Disease.

Groups \ Parameters		Protein Status (g/l)		
		Protein	**Albumin**	**Globulin**
Healthy rats fed on basal diet (control - ve)		7.43 [a] ± 0.152	4.30 [a] ± 0.264	3.13 [a] ± 0.115
Acute liver disease in rats fed on a	basal diet (control + ve)	6.30 [e] ± 0.100	3.77 [b] ± 0.351	2.53 [b] ± 0.251
	diet containing 1% choline	6.46 [d e] ± 0.152	4.00 [a b] ± 0.200	2.47 [b] ±0.057
	diet containing 2% choline	7.13 [a b] ± 0.305	4.10 [a b] ± 0.264	3.03 [a] ±0.152
	diet containing 5% ZS-C leaves	6.67 [c d] ± 0.230	3.97 [a b] ± 0.208	2.70 [b] ± 0.173
	diet containing 7.5% ZS-C Leaves	6.93 [b c] ± 0.152	3.83 [ab] ± 0.208	3.10 [a] ± 0.100
	diet containing 1% choline and 5% ZS-C leaves	6.93 [b c] ± 0.230	3.87 [a b] ± 0.115	3.06 [a] ± 0.115
	diet containing 2% choline and 7.5% ZS-C leaves	7.07 [b] ± 0.115	4.00 [a b] ± 0.100	3.07 [a] ± 0.208

ZS-C: Zizyphus Spina-christileaves

Mean values in each column with same letters are not significantly different.

In this respect, **(Ogeturk et al., 2004)** found higher serum AST, ALT, bilirubin, ALP, gamma-GT, and iron levels in CCl4-treated rats compared to controls, while urea, total protein, and albumin levels were lower.

Khaleel et al. (2021) discovered that an ethanolic leaf extract of *Ziziphus Spina-christi* significantly increased serum protein levels and total protein concentration in rats, possibly due to saponins increasing gut protein absorption, suggesting Z. *Spina-christi*may also improve serum albumin levels. Natural antioxidants in Sidr Fermented Fruit SFP prevent oxidative damage, cytotoxicity, and inhibitory effects from malondialdehyde accumulation. These antioxidants, including flavonoids, phenolics, minerals, and vitamins, act as superoxide scavengers, suppressing reactive oxygen species and uric acid formation **(Cheng et al., 2021; Zandiehvakili and Khadivi, 2021 and Lin et al., 2015).**

Zhang et al. (2018) found that choline supplementation significantly increased serum albumin levels in rats with liver damage, improved liver function, and reduced oxidative stress. On the other hand, **Zhang et al. (2017)** choline supplementation in mice with kidney damage increased serum albumin levels, improved kidney function, and reduced inflammation, suggesting potential benefits for human serum protein levels.

Effect of Some Levels from *Zizyphus Spina-christi* Leaves and Choline on Antioxidants Enzymesof Serum Rats Suffering from Acute Liver Disease.

Table (4) demonstrated the impact of two levels of *Zizyphus Spina*-christi leaves and choline on the antioxidant enzymes in the serum of rats with acute liver disease.Injecting rats fed on a basal diet with CCl4 to induce acute liver decreased the mean value of antioxidants enzymes including (Catalase "CAT", Superoxide dismutase "SOD", Glutathione "GSH" and Glutathione peroxidase "GPx"), as compared to non-injected rats which fed on the same diet.

Feedingrats which were suffering from acute liver disease with two levels of choline, *Zizyphus Spina-christi*leaves, and their combination led to a significant increase ($p \leq 0.05$) in all antioxidants enzymes. Diet containing 2% choline and 7.5% ZS-C leaves recorded the best results in catalase, because this treatment showed non-significant change in this parameter, as compared to healthy rat "control –ve group). Groups of rats which treated with diets containing 2%choline, 7.5% ZS-C Leaves, 1% choline and 5% ZS-C leaves, and 2% choline and 7.5% ZS-C leaves showed the best results in Superoxide dismutase (SOD), because these group showed non-significant differences in SOD, as compared to the negative control group. The best results in GSH recorded for the group treated with Diet containing 2% choline and 7.5% ZS-C leaves and the group treated with diet containing 1% choline and 5% ZS-C leaves, followed by the group treated with diet containing 7.5% ZS-C Leaves, respectively. Diet

containing (2% choline and 7.5% ZS-C leaves, and 7.5% ZS-C Leaves) improved Glutathione peroxidase "GPx, as compared to the other treated groups.

In this respect, **(Hadayat Ullah et al., 2020)** investigated the effects of CCL4 on oxidative stress and antioxidant enzymes in mouse liver. The mice were exposed to CCL4 for 4 weeks, and then their livers were harvested and analyzed for levels of oxidative stress markers and antioxidant enzymes. The results of the study showed that CCL4 exposure significantly increased levels of oxidative stress markers, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). CCL4 exposure also significantly decreased levels of antioxidant enzymes, such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT).

Mercury intoxication in rats increased lipid peroxidation and nitric oxide levels, leading to oxidative stress and glutathione depletion, but ZCE treatment attenuated these levels and restored them to control **(Ramadan et al., 2021)**.

Table (4): Effect of Some Levels from *Zizyphus Spina-christi*Leaves and Choline on Antioxidants Enzymesof Serum Rats Suffering from Acute Liver Disease.

Groups / Parameters		Catalase (CAT) U/l	Superoxide dismutase (SOD) U/l	Glutathione GSH mg/l	Glutathione peroxidase (GPx) U/ml
Healthy rats fed on basal diet (control - ve)		82.33 ^{a} ± 2.08	921.00 ^{a} ± 61.098	4.12 ^{a} ± 0.278	55.00 ^{a} ± 2.645
Acute liver disease in rats fed on a	basal diet (control + ve)	37.00 ^{d} ± 2.645	487.66^{c} ± 10.785	1.88 ^{f} ± 0.102	22.00 ^{d} ± 1.732
	diet containing 1% choline	52.33 ^{c} ± 4.509	800.67^{b} ± 58.483	3.10 ^{e} ± 0.065	24.33 ^{d} ± 1.527
	diet containing 2% choline	72.33 ^{b} ± 3.214	912.00 ^{a} ± 16.822	3.38 ^{d} ± 0.015	36.00^{bc} ± 3.00
	diet containing 5% ZS-C leaves	72.67 ^{b} ± 2.516	799.67^{b} ± 4.163	3.60 ^{c} ± 0.086	25.67 ^{d} ± 1.527
	diet containing 7.5% ZS-C Leaves	76.33 ^{b} ± 4.725	907.00 ^{a} ± 4.358	3.75 $^{b\,c}$ ± 0.071	40.00 ^{b} ± 3.505
	diet containing 1% choline and 5% ZS-C leaves	74.00 ^{b} ± 2.00	916.33 ^{a} ± 8.504	3.87^{b} ± 0.036	31.00 ^{c} ± 3.000
	Diet containing 2% choline and 7.5% ZS-C leaves	78.00^{ab} ± 3.00	919.33 ^{a} ± 7.371	3.84 ^{b} ± 0.133	36.33 ^{b} ±4.725

ZS-C: Zizyphus Spina-christileaves

Mean values in each column with same letters are not significantly different.

The oral administration of *Zizyphus Spina*-christi (ZSC) has not only reinstated normal malondialdehyde levels but has also preserved the control activities of endogenous antioxidants, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH). Additionally, ZSC has shown efficacy in reducing the expression of α-smooth muscle actin and the deposition of types I and III collagen in rats with CCl4-induced injuries. Texture analysis of microscopic images, coupled with fibrosis index calculation, has indicated an enhancement in the quality and quantity of type I collagen distribution following ZSC extract administration. These findings suggest that ZSC administration may hold promise for both the treatment and prevention of hepatic fibrosis **(Amin and Mahmoud-Ghoneim., 2009).**

ZSCF powder at 2.5, 5, 10, and 15% of basal diet for 6 weeks restored malondialdehyde levels, and controlled endogenous antioxidants like SOD and GSH. It protects the liver against CCL4-induced oxidative damage in rats, possibly due to its antioxidant and free radical scavenger effects **(Yossef et al., 2011)**. On the other hand, **(El Rabey et al., 2013 and Al-Sieni, 2014)** reported that induced hyperlipidemia reduced antioxidant enzymes (catalase, SOD and Glutathione reductase) and increased lipid peroxide in rats; *Ziziphus Spina-christi*leaves improved these parameters.

Ganesan et al., (2011) found that betaine, an antioxidant, significantly prevents stress-induced changes in protein and corticosterone levels, counteracts lipid peroxidation, and maintains the antioxidant defense system in lymphoid tissues, possibly due to its antioxidant properties. Researchers found

choline and betaine protect against oxidative stress, improve cognitive function, and increase antioxidant enzyme expression (CAT and SOD) in aged mice **(Zeisel et al., 2006).**

Histopathological examination of liver:

The liver of rats from a normal control group, fed a basal diet, showed a normal histological structure of the hepatic lobule(Photo 1).The liver of rats with acute liver disease, fed a basal diet, showed degeneration, intravascular permeation with inflammatory cells, congestion of blood vessels, portal edema, and infiltration of inflammatory cells(Photo 2, 3 & 4).The liver of an acute liver disease group fed a basal diet containing 1% choline showed activation of Kupffer cells and thickening of the bile duct wall(Photo 5 & 6).The liver of rats with acute liver disease fed on a diet containing 2% choline showed activation of Kuphher cells, portal triad fibroplasia, and newly formed bile ductless(Photo 7 and 8).The liver of rats with acute liver disease fed on diet containing 5% ZS-C leaves showed slight activation of Kupffer cells, hepatocellular vacuolar degeneration, and sinusoidal leukocytosis (Photo 9, 10 & 11).The study found that rats with acute liver disease fed 7.5% ZS-C leaves showed slight hepatocellular vacuolar degeneration and focal hepatic necrosis due to inflammatory cell infiltration (Photo 12, 13 & 14).The liver of rats with acute liver disease fed a diet containing 1% choline and 5% ZS-C leaves showed only slight activation of Kupffer cells(Photo 15 & 16).The liver of rats with acute liver disease, fed a diet containing 2% choline and 7.5% ZS-C leaves, showed slight hepatocellular vacuolar degeneration and central vein congestion(Photo 17 & 18).

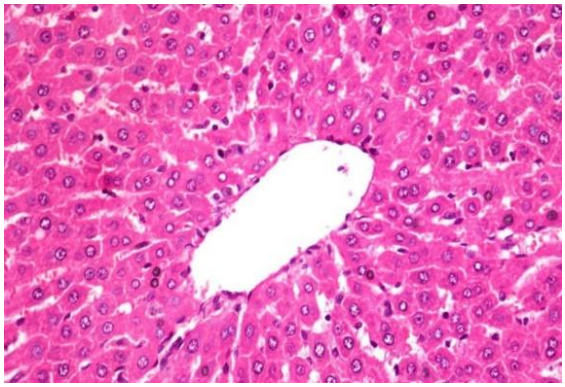

Photo 1: Photomicrograph of the liver of a rat from the normal control group fed on a basal diet showing the normal histological structure of the hepatic lobule (H & E X 400).

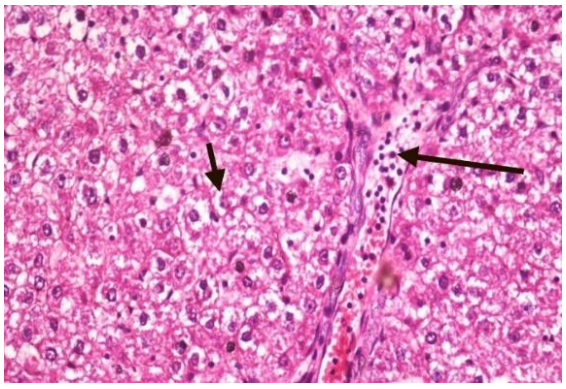

Photo 2: Photomicrograph of the liver of acute liver disease rats fed on a basal diet showing hepatocellular vacuolar degeneration (short arrow) and intravascular permeation with inflammatory cells (long arrow) (H & E X 400).

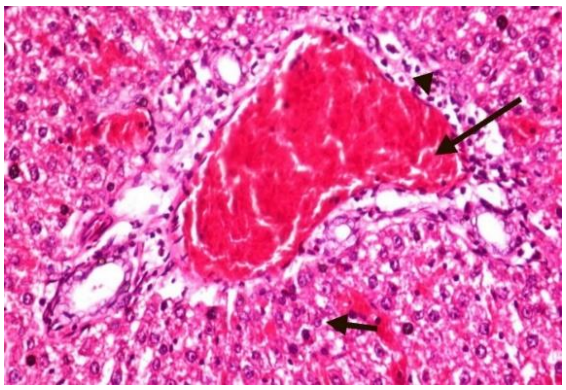

Photo 3: Photomicrograph of acute liver disease rats fed on a basal diet showing hepatocellular vacuolar degeneration (short arrow), congestion of hepatoportal blood vessels (long arrow), and portal infiltration with inflammatory cells (arrow head) (H & E X 400).

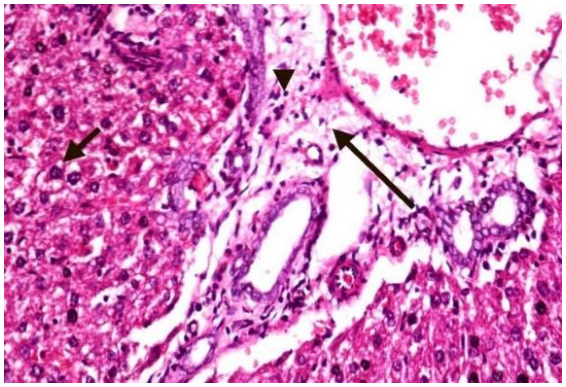

Photo 4: Photomicrograph of the liver of acute liver disease rats fed on a basal diet showing hepatocellular vacuolar degeneration (short arrow), portal edema (l arrow), and inflammatory cells (arrow head) (H & E X 400).

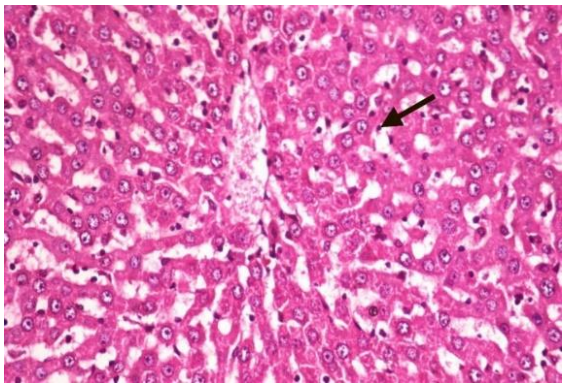

Photo 5: Photomicrograph of the liver of acute liver disease group fed on a diet containing 1% choline showing Kupffer cell activation (arrow) (H & E X 400).

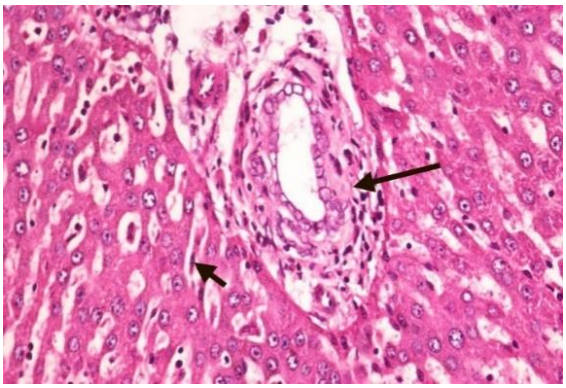

Photo 6: Photomicrograph of the liver of an acute liver disease group fed on a diet containing 1% choline showing Kupffer cells activation (short arrow) and thickening in the wall of the bile duct (long arrow) (H & E X 400).

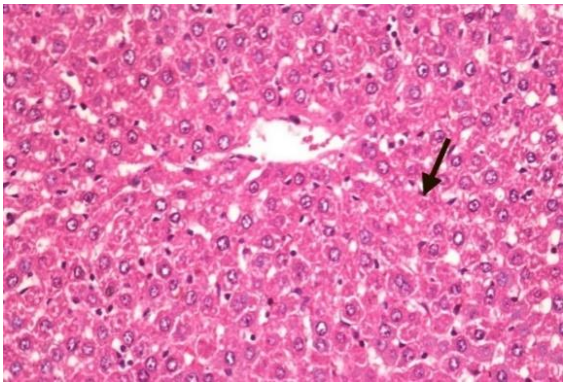

Photo 7: Photomicrograph of the liver of acute liver disease rats fed on a diet containing 2% choline showing slight Kupffer cell activation (arrow) (H & E X 400).

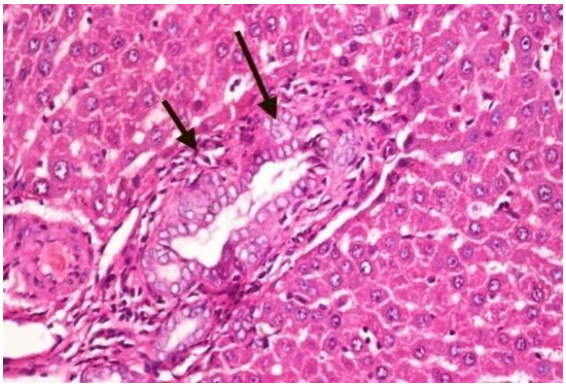

Photo 8: Photomicrograph of the liver of acute liver disease rats fed on a diet containing 2% choline, showing fibroplasia in the portal triad (short arrow) and newly formed bile ductuoles (long arrow) (H & E X 400).

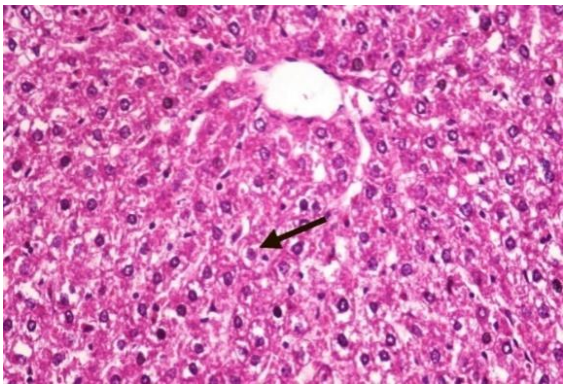

Photo 9: Photomicrograph of the liver of acute liver disease rats fed on a diet containing 5% ZS-C leaves showing slight hepatocellular vacuolar degeneration (arrow) (H & E X 400).

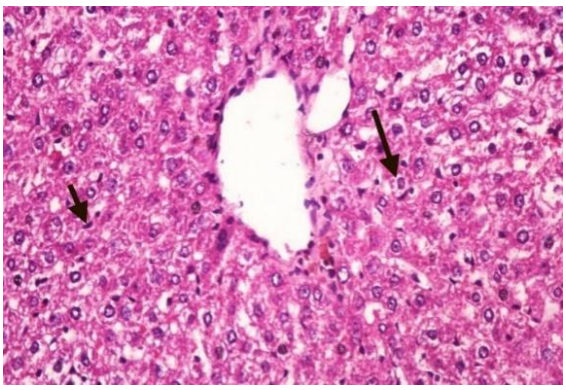

Photo 10: Photomicrograph of the liver of acute liver disease rats fed on a diet containing 5% ZS-C leaves showing slight Kupffer cell activation (short arrow) and slight hepatocellular vacuolar degeneration (long arrow) (H & E X 400).

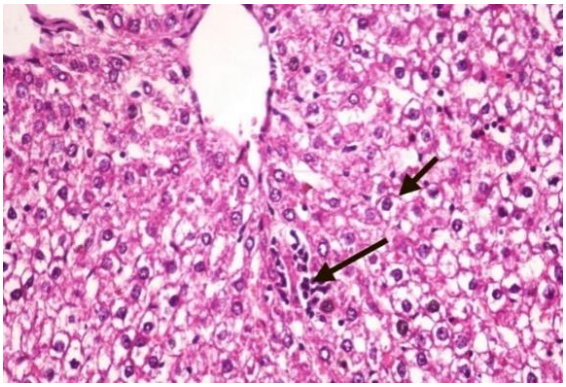

Photo 11: Photomicrograph of the liver of acute liver disease rats fed on a diet containing 5% ZS-C leaves showing hepatocellular vacuolar degeneration (short arrow) and sinusoidal leukocytosis (long arrow) (H& E X 400).

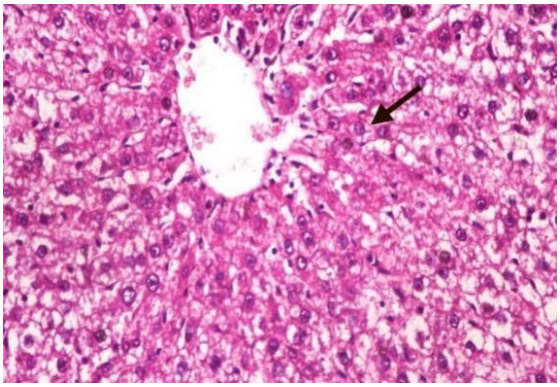

Photo 12: Photomicrograph of the liver of acute liver disease rats fed on a diet containing 7.5% ZS-C leaves showing slight hepatocellular vacuolar degeneration (arrow) (H & E X 400).

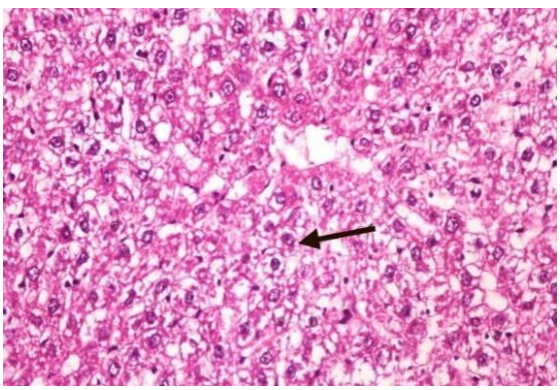

Photo 13: Photomicrograph of the liver of acute liver disease rats fed on a diet containing 7.5% ZS-C leaves showing slight hepatocellular vacuolar degeneration (arrow) (H & E X 400).

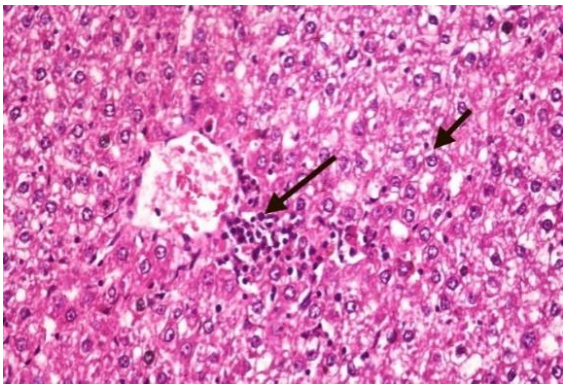

Photo 14: Photomicrograph of the liver of acute liver disease rats fed on a diet containing 7.5% ZS-C leaves showing slight hepatocellular vacuolar degeneration (short arrow) and focal hepatic necrosis associated with inflammatory cell infiltration (long arrow) (H & E X 400).

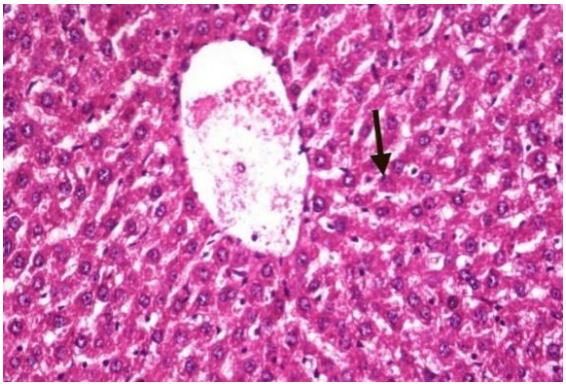

Photo 15: Photomicrograph of the liver of acute liver disease rats fed on a diet containing 1% choline and 5% ZS-C leaves showing slight Kupffer cell activation (arrow) (H & E X 400).

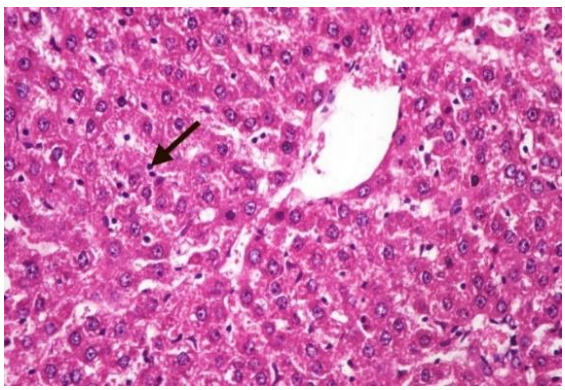

Photo 16: Photomicrograph of the liver of acute liver disease rats fed on a diet containing 1% choline and 5% ZS-C leaves showing slight Kupffer cell activation (arrow) (H & E X 400).

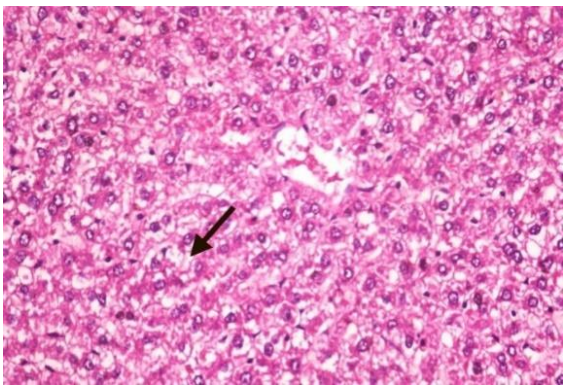

Photo 17: Photomicrograph of the liver of acute liver disease rats fed on a diet containing 2% choline and 7.5% ZS-C leaves showing slight hepatocellular vacuolar degeneration (arrow) (H & E X 400).

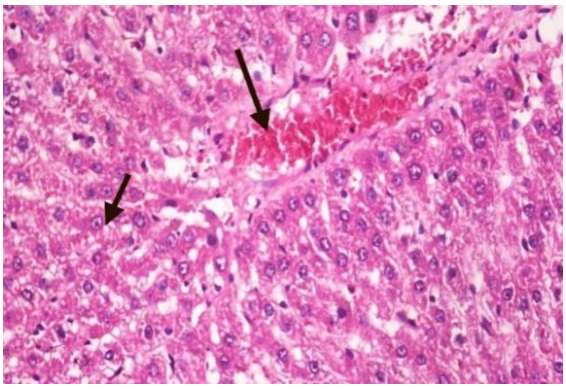

Photo 18: Photomicrograph of the liver of acute liver disease rats fed on a diet containing 2% choline and 7.5% ZS-C leaves showing slight hepatocellular vacuolar degeneration (short arrow) and congestion of the central vein (long arrow) (H & E X 400).

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