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Effect of *Zizyphus Spina-Christi* Leaves and Choline on glucose levels and kidney impairment in rats with induced Acute Liver Disease

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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 combinations on nutritional evaluation, some biochemical analysis, and histopathological examination in the kidneys of rats suffering from acute liver disease. Forty-eight male albino rats were used in this study. The rats were divided into two main groups, as follows: **The first main group of six rats was fed** a basal diet (as a negative control group). **The second main group (42 rats)** was injected with CCl4 in paraffin oil (50% v/v, 4 ml/kg) by subcutaneous injection to induce acute damage in the liver. The rats in the second main group were divided into7 subgroups (6 rats each) 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. The results indicated that injected rats with CCl4 increased serum glucose, uric acid, urea nitrogen, creatinine, cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol, while decreasing feed intake, serum HDL-cholesterol, 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 kidney as compared to non-treated rats. **Conclusion**: *Ziziphus spina-Christi* leaves, choline, and their combinations can be used to reduce the side effects of acute liver diseases.

Keywords: Acute liver disease; *Ziziphus Spina-Christi* leaves; Choline; kidney function; glucose and lipid profile

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INTRODUCTION

Liver disease affects over 10% of the global population, with cirrhosis and cancer being the most common fatal end-stage **(Muriel, 2017).** Environmental pollution and toxic chemicals, like CCl_4, cause toxic radical species genesis, lipid peroxidation, and cell damage through exposure in drinking water, air, and industrial sites **(Ekpo et al., 2020).** CCl_4 induces hepatotoxicity in rats using chemical hepatotoxin **(Zhang et al., 2017).**

Nabaq (*Zizyphus spina-christi* L) fruit has a rich composition of bioactive components such as phenolic, flavonoids, dietary fiber, minerals and vitamins **(Atwaa et al., 2021).**

Zizyphus spina-christi prevents fibrosis due to its antioxidant actions, and alleviates fibrosis through reducing the depositions of both actin and collagen fibers. ZSC may therefore offer an attractive drug target for an array of fibrotic diseases including degenerative chronic diseases, i.e. renal fibrosis, cardiac diseases and hepatic cirrhosis **(Amin and Ghoneim., 2009).**

The increase in body weight gain and feed intake of poultry has been found by many researchers after the addition of dietary choline, Choline has a crucial importance in respect to the brain development in children and the development of the longevity functions **(Önel et al, 2017).**

In cases of choline insufficiency, diseases such as liver fattening, renal impairment, and even cancer may emerge. Studies have shown that problems in liver, kidneys, and pancreas and disorders of memory and growth might be encountered in chronic choline insufficiency. The results obtained showed that choline has an important potential in the control of fatty liver. Choline can be referred to as a lipotropic agent due to its function of acting on fat metabolism by hastening removal or decreasing deposition of fat in liver **(Rozenboim et al., 2016).**

Therefore, the present study was carried out to assess the effects of some levels from *Ziziphus Spina –Christi* leaves and choline on glucose levels and kidney impairment in rats induced Acute Liver Disease.

MATERIALS AND METHODS

Materials: Casein, vitamins, minerals, cellulose, CCl_4, and choline chloride were obtained from El Gomhoriya Company, Cairo, Egypt. Starch and corn oil were obtained from the local market. *Zizyphus spina-christi* was obtained from the local market of Agricultural Herbs and Medicinal plants in Cairo, Egypt.

Rats: Forty-eight male albino rats of the Sprague Dawley strain, weighing 180 ± 10g, were purchased from the laboratory of the animal colony, Ministry of Health and Population, Helwan, Cairo, Egypt.

Kits: 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)**, forty-eight male albino rats of the Sprague-Dawley strain weighing 180 ± 10g were kept in individual stainless-steel cages under hygienic conditions and fed one week on a basal diet ad libitum for adaptation in the biological studies lab of the Faculty of Home Economics, Helwan University. After a period of adaptation to the basal diet (7 days), the rats were divided into two main groups. The first main group (6 rats) was fed a basal diet as a control negative group. The second main group (42 rats) was treated with CCl4 in single

dose of paraffin oil (50% v/v, 4 ml/kg) by subcutaneous injection to induce acute damage in the liver **(Jayasekhar et al., 1997).** After confirming the induction of the disease by determining serum glucose, uric acid, urea nitrogen, creatinine, HDL-c, LDL-c,VLDL-c, triglycerides and cholesterol in the first and second main groups, the second main group was divided into 7 subgroups (6 rats each) 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.

At the end of the experiment, the rats were fasted overnight, and then the rats were anaesthetized and sacrificed. Blood samples were collected from the aorta of all rats. The blood samples were centrifuged and serum was separated to estimate some biochemical parameters, i.e., glucose **(Trinder., 1969)**, uric acid **(Fossati *et al.*, 1980)**, urea nitrogine **(Patton and crouch., 1977)**, creatinine **(Bartels and Bohmer., 1971)**, HDL-c **(Burstein, 1970)**, LDL-c **(Friedwald *et al.*, 1972)**, VLDL-c **(Friedwald *et al.*, 1972)**, triglycerides **(Fossati and Prencipe., 1982)** and cholesterol **(Allain *et al.*, 1974)**.

Specimens from kidney tissues were taken immediately after sacrificing animals and fixed in a 10% buffered neutral formalin solution. The fixed specimens were then trimmed, washed, and dehydrated, imbedded in paraffin, cut in sections of 46 microns' thickness, and stained with haematoxylin and eosin stain, according to **Sheehan and Hrapchak (1980).**

The results of the biological evaluation of each group were statistically analysed (mean ± standard deviation and one-way ANOVA test) by using the SPSS package and compared with each other using the suitable test (least significant differences at $P< 0.05$) according to **Sendecor and Cochran (1979)**.

RESULTS AND DISCUSSION

Effect of Some Levels of *Zizyphus Spina-Christi* Leaves and Choline on Feed Intake and Glucose of Rats Suffering from Acute Liver Disease.

The study examines the impact of *Zizyphus Spina-Christi* leaves, choline, and their combination on glucose in rats with acute liver disease, as shown in Table (1). The study found that control negative rats consumed 19.166 g/each rat/day of a basal diet, while rats with induced 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 data in this table revealed that the mean value of feed intake in all tested groups showed non-significant differences as compared to the negative control group. On the other hand, these treatments showed a significant increase ($p \leq 0.05$) in this parameter as compared to the positive control group.

The study found that injecting rats with CCl4 to induce acute liver disease significantly increased the mean value of serum glucose, by about 88.137% in the positive control group compared to that of the negative control group. The results in this Table revealed that treating acute liver disease groups with *Zizyphus Spina-Christi* leaves, choline, and their combination significantly reduced the mean value of serum glucose compared to the positive control group. Serum glucose decreased gradually with increasing the level of choline in the diet, while a non-significant change in serum glucose was observed between acute liver disease groups that were treated with 5% and 7.5% "ZS-C leaves. On the other hand, treating acute liver disease rats with high and low levels of the mix of choline and ZS-C leaves caused a non-significant difference between them.

The highest decrease in serum glucose was observed in acute liver disease groups that were treated with a diet containing 7.5% ZS-C Leaves, followed by a diet containing 1% choline and 5% ZS-C leaves, 5% ZS-C leaves, and 2% choline, respectively. In this respect, **Khan et al., (2015)** reported that glucose levels increased in animals treated with CCl4 as a result of decreased pancreatic secretion of insulin from β-cells of Langerhans islets because of fatty changes in the cells of the islets resulting from the toxicity of CCl4.

Experimental rats treated with hydro-alcoholic extract of *Ziziphus Jujuba* leaves showed hypoglycemic effect, reduced lipids, and cholesterol **(Alsayari and Wahab., 2021).**On the other hand, **(Benammar and Baghdad, 2014)** reported that root and leaf extracts of *Zizyphus lotus* extracts show antioxidant and anti-diabetic effects in diabetic rats. **Alsayari and Wahab., (2021)** reported also *Ziziphus Spina-christi* effectively reduces insulin and blood glucose levels in-diabetic control group, type-I (insulin-dependent) and type-II (non-insulin-dependent).

Table (1): Effect of Some Levels from *Zizyphus Spina-Christi* Leaves and Choline on Serum Glucose of Rats Suffering from Acute Liver Disease.

Groups \ Parameters		Glucose mg/dl	Feed Intake g/day/each rat
Control negative rats fed on basal diet (control - ve)		78.666 ^{d} ± 3.214	19.166 ^{a} ± 0.288
Acute liver disease rats fed on a	basal diet (control + ve)	148.000^{a} ± 7.937	17.266^{b} ± 0.680
	diet containing 1% choline	98.666^{b} ± 2.081	19.166 ^{a} ± 0.288
	diet containing 2% choline	91.000 ^{c} ± 2.645	18.986 ^{a} ± 0.450
	diet containing 5% ZS-C leaves	88.000 ^{c} ± 3.464	18.796 ^{a} ± 0.270
	diet containing 7.5% ZS-C Leaves	86.333 ^{c} ± 5.507	18.833 ^{a} ± 0.152
	diet containing 1% choline and 5% ZS-C leaves	87.000 ^{c} ± 2.645	19.00 ^{a} ± 0.556
	diet containing 2% choline and 7.5% ZS-C leaves	93.333 $^{b\,c}$ ± 2.516	19.200 ^{a} ± 0.200

ZS-C: Zizyphus Spina-Christi leaves

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

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 weight gains and feed intake increased as dietary choline increased from 0-0.75 g/kg. **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.

Betaine improves adipokine levels, insulin sensitivity, endoplasmic stress, fatty acid oxidation, and mitochondrial function **(Zhou et al.,2015).** Betaine improves insulin resistance in mice fed a high-fat diet **(Du et al.,2018).** Betaine prevents isoprenaline-induced myocardial dysfunction through antioxidant and mitochondrial protection **(Ganesan and Anandan.,2009).**

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

Injecting rats fed on a basal diet with CCl_4 to induce acute liver diseases increase $P \leq 0.05$ in serum uric acid, as compared to non-injected rats fed on the same diet. The mean value of serum uric acid increased in the positive control group by about 72.608% compared to that of the negative control group.

Treatment of acute liver disease groups with two levels of choline, 1% choline induced a non-significant difference in the mean value of serum uric acid as compared to the positive control group, while feeding acute liver disease rats with a basal diet containing 2% choline caused asignificant decrease ($p \leq 0.05$) in the mean value of serum uric acid. The mean value of serum uric acid decreased in the group fed on a basal diet containing 2% choline by about 16.12% compared to that of the positive control group.

Treating acute liver disease rats with 5% ZS-C leaves led to a non-significant difference in the mean value of serum uric acid, while treating acute liver disease rats with 7.5% caused a significant decrease ($p \leq 0.05$) in the mean value of serum uric acid, as compared to the positive control group. Feeding rats suffering from an acute liver disease on a basal diet containing 7.5% ZS-C leaves decreased the mean value of serum uric acid by about 11.083% compared to that of the positive control group.

The best results in serum uric acid were recorded for the group fed on the combination of choline and ZS-C leaves with levels of 2% choline and 7.5% ZS-C leaves, followed by the group treated with a combination of 1% choline and 5% ZS-C leaves and 2% choline, respectively.

Injected rats with CCl4 to induce acute liver disease and fed on basal diet caused significant increase $P \leq 0.05$ in serum urea nitrogen, as compared to non-injected rats which fed on the same diet. The mean value of serum urea nitrogen increased in the positive control group by about 50% than that of the negative control group.

The treatment of acute liver disease rats with choline and Sidr leaves and their combination showed a significant decrease $p \leq 0.05$ in the mean value of serum urea nitrogen in all treated groups, as compared to the positive control group. Also, the data revealed that non-significant changes in the mean value of serum urea nitrogen were observed between the groups treated with (5% vs. 7.5% ZS-C leaves), and their combination between choline and ZS-C leaves. Rats suffering from acute liver diseases and fed on a basal diet containing 2% choline plus 7.5% ZS-C leave recorded the best result in serum urea nitrogen decreased about 29.638% compared to that of the positive control group.

Treated rats with CCl_4 and fed on a basal diet caused a significant increase $P \leq 0.05$ in serum creatinine, as compared to non-treated rats who fed on the same diet, the mean value of serum creatinine increased in the positive control group by about 46% compared to of the negative control group.

Feeding rats who were suffering from an acute liver disease on a basal diet containing 1% choline induced a non-significant difference in the mean value of serum creatinine as compared to the positive control group, while feeding acute liver disease rats with a basal diet containing 2% choline caused a significant decrease ($p \leq 0.05$) in the mean value of serum creatinine by about 19.178% as compared to that of the positive control group.

Feeding acute liver disease rats on abasal diet containing 5% ZS-C leaves led to a non-significant difference in the mean value of serum creatinine, while feeding acute liver disease rats on adiet containing 7.5% ZS-C leaves caused a significant decrease ($p \leq 0.05$) by about 21.917% compared to that of the control positive group.

Treating acute liver disease rats with a basal diet containing the combination of choline and ZS-C leaves with levels of 1% choline and 5% ZS-C leaves recorded non-significant differences as compared to the positive control group. On the other hand, feeding rats with acute liver disease on a basal diet containing (2% choline and 7.5% ZS-C leaves) decreased the mean value of serum creatinine significantly $p \leq 0.05$, as compared to the positive control group. Feeding rats suffering from acute liver diseases a basal diet containing (2% choline and 7.5% ZS-C leaves) decreased the mean value of serum creatinine by about 24.657% compared to that of the positive control group.

The data presented in this Table (2) showed that feeding rats suffering from acute liver disease on a basal diet containing a high level of combination between (choline and ZS-C leaves) or (ZS-C leaves) and/or (choline) recorded the best results in serum creatinine.

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

Groups / Parameters		Kidney Functions(mg/dl)		
		Uric acid	Urea nitrogen	Creatinine
Control negative rats fed on basal diet (control - ve)		2.30 ^{d} ± 0.264	24.00 ^{d} ± 2.00	0.50 ^{c} ± 0.100
Acute liver disease rats fed on a	basal diet (control + ve)	3.97 ^{a} ± 0.152	36.00 ^{a} ± 2.00	0.73 ^{a} ± 0.057
	diet containing 1% choline	3.70 $^{a b}$ ± 0.100	31.33 ^{b} ± 2.309	0.63 $^{a b c}$ ± 0.057
	diet containing 2% choline	3.33 ^{b} ± 0.251	27.67 ^{c} ± 2.081	0.59 $^{b c}$ ± 0.049
	diet containing 5% ZS-C leaves	3.60 $^{a b}$ ± 0.200	28.67 $^{b c}$ ± 1.154	0.67 $^{a b}$ ± 0.115
	diet containing 7.5% ZS-C Leaves	3.53 ^{b} ± 0.208	27.33 ^{c} ± 1.527	0.57 $^{b c}$ ± 0.057
	diet containing 1% choline and 5% ZS-C leaves	3.36 ^{b} ± 0.208	27.33 ^{c} ± 1.154	0.62 $^{a b c}$ ± 0.081
	diet containing 2% choline and 7.5% ZS-C leaves	2.87 ^{c} ± 0.251	25.33 $^{c d}$ ± 1.527	0.55 $^{b c}$ ± 0.050

ZS-C: Zizyphus Spina-Christi leaves

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

In this respect, **(Makni et al., 2012)** found CCl_4 significantly inducing renal disorder, oxidative damage, and DNA fragmentation, with increased levels of creatinine, urea, and MDA. **Ganie et al., (2011)** reported that CCl_4 exposure causes tissue injury in various organs such as the kidney, heart, brain, testis, and lung, due to excessive free radical production.

Khaleel et al., (2021) reported that creatinine, urea, and uric acid levels may indicate renal dysfunction. Leaf extract did not show significant differences in creatinine, urea, and uric acid levels in rats, suggesting no negative effect on kidneys.

Ziziphus spina-christi leaf extract ZSCLE can prevent mercury-induced kidney alterations, inhibit creatinine, urea and reduce antidiabetic effects in rat models of diabetes **(Dkhil et al., 2018 and Jarald et al., 2009)**. Also, (**Rafa et al., 2019**) reported that HgCl2-treated mice's kidney tissue showed severe damage, but normal histology was preserved during ZSCLE therapy.

Choline insufficiency leads to liver fattening, heart disease, bone anomalies, and kidney dysfunction **(Phillips, 2012)**. **Kratzing et al., (1970)** found that choline deficiency in rats caused increased levels of uric acid, urea, and creatinine in the blood.

Betaine can protect rats from acute renal failure ARF caused by myocardial infarction. The study found that betaine reduced oxidative stress, inflammation, and cell death in the kidneys of rats with myocardial infarction-induced ARF. Betaine also improved kidney function in rats with myocardial infarction-induced ARF **(Ghartavol et al., 2019)**.

Effect of Some Levels from *Zizyphus Spina-Christi* Leaves and Choline on Serum Cholesterol and Triglycerides in Rats Suffering from Acute Liver Disease.

The study examines the impact of *Zizyphus Spina-Christi* leaves, choline, and their combination on Cholesterol and Triglycerides in rats with acute liver disease, as shown in Table (3). The study found that injecting rats with CCl4 to induce acute liver disease significantly increased the mean value of serum cholesterol and triglycerides in the positive control group increased by about 84.238%, 99.255%, respectively than that of the negative control group.

All treating groups with two levels of choline, or two levels from ZS-C leaves, and or their combinations showed a significant decrease $p \leq 0.05$ in serum cholesterol and triglycerides, as compared to the positive control group.

The data presented in this Table revealed that the mean value of serum cholesterol of the groups which were treated with low levels of (choline, ZS-C leaves, and their combinations) recorded non-significant change between them. While the treating groups that were suffering from acute liver disease with 7.5% ZS-C Leaves or the combination between (2% choline and 7.5% ZS-C leaves) showed a significant decrease $p \leq 0.05$ in the mean value of serum cholesterol, as compared to the group that treated with 2% choline.

Groups of rats that were fed on a diet containing 7.5% ZS-C leaves or a diet containing a combination between (2% choline and 7.5% ZS-C leaves) recorded the best results, because these groups showed a significant decrease in this parameter, as compared to the other treated groups.The data presented in this table showed non-significant differences in the mean value of serum triglycerides in all treated groups, except the groups treated with (1% choline and 5% ZS-C leaves), as compared to the negative control group.

Table (3): Effect of Some Levels from *Zizyphus Spina-Christi* Leaves and Choline on Serum Cholesterol, Triglycerides of Rats Suffering from Acute Liver Disease.

Groups \ Parameters		Cholesterol	Triglycerides
		mg/dl	
Control negative rats fed on basal diet (control - ve)		103.67 [e] ±3.785	44.33 [c] ± 2.081
Acute liver disease rats fed on a	basal diet (control + ve)	191.00 [a] ±7.810	88.33 [a] ± 7.371
	diet containing 1% choline	135.67 [b] ± 1.527	56.33 [b] ± 4.725
	diet containing 2% choline	124.67 [c] ± 2.309	52.00 [b c] ± 4.582
	diet containing 5% ZS-C leaves	141.66 [b] ± 6.027	56.00 [b] ± 3.605
	diet containing 7.5% ZS-C Leaves	116.33 [d] ± 2.081	45.33 [c] ± 3.055
	diet containing 1% choline and 5% ZS-C leaves	135.00 [b] ± 3,785	52.67 [b c] ± 5.033
	diet containing 2% choline and 7.5% ZS-C leaves	113.67 [d] ± 3.785	45.67 [c] ± 3.511

ZS-C: Zizyphus Spina-Christi leaves
Mean values in each column with same letters are not significantly different.

Effect of Some Levels from *Zizyphus Spina-Christi* Leaves and Choline on Serum Lipoprotein-cholesterol in Rats Suffering from Acute Liver Disease.

The effects of two levels from *zizyphus spina-christi* leaves (ZS-c leaves), choline, and their combination on serum lipoprotein-cholesterol including (high density lipoprotein-cholesterol HDL-c, low and very low-density lipoprotein-cholesterol LDL-c & VLDL-c in rats suffering from acute liver disease are presented in Table (4).

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

Groups \ Parameters		Serum Lipoprotein (mg/dl)		
		HDL-c	LDL-c	VLDL-c
Control negative rats fed on basal diet (control - ve)		44.00 [a] ± 2.00	50.80 [f] ± 3.903	8.87 [c] ± 0.416
Acute liver disease rats fed on a	basal diet (control + ve)	29.00 [d] ± 4.358	144.33 [a] ± 5.707	17.67 [a] ± 1.474
	diet containing 1% choline	35.33 [b c] ± 3.055	89.07 [c] ± 4.110	11.27 [b] ± 0.945
	diet containing 2% choline	37.00 [b c] ± 1.00	77.27 [d] ± 0.986	10.40 [b c] ± 0.916
	diet containing 5% ZS-C leaves	33.00 [c d] ±2.645	97.47 [b] ± 2.837	11.20 [b] ± 0.721
	diet containing 7.5% ZS-C Leaves	38.00 [b c] ± 2.00	69.27 [e] ± 1.331	9.07 [c] ± 0.611
	diet containing 1% choline and 5% ZS-C leaves	39.33 [a b] ± 4.163	85.13 [c] ± 5.021	10.53 [b c] ± 1.01
	diet containing 2% choline and 7.5% ZS-C leaves	40.33 [a b] ± 1.527	64.20 [e] ± 1.78	9.13 [c] ± 0.702

ZS-C: Zizyphus Spina-Christi leaves
Mean values in each column with same letters are not significantly different.

High Density Lipoprotein-cholesterol "HDL-c" (mg/dl):

The data in Table (4) showed that injected rats with CCl-4 to induce acute liver disease in rats "control +ve group) caused a significant decrease $p \leq 0.05$ in serum HDL-c, as compared to non-injected rats "control –ve group" The mean value of serum HDL-c in the positive control group decreased by about 34.09% than that of the negative control group.

All treated groups which were suffering from acute liver diseases with the two levels of choline, ZS-C, and their combination showed a significant increase in the mean value of serum HDL-c, except the group of rats treated with 5% ZS-C leaves, as compared to the positive control group.

The results in this Table revealed that feeding rats were suffering from acute liver diseases with diets containing a combination between "1% choline and 5% ZS-C leaves" and "2% choline and 7.5% ZS-C leaves" led to non-significant differences in the mean value of serum HDL-c, as compared to the negative control group "healthy rat". These treatments showed the best results in HDL-c.

Low Density Lipoprotein-cholesterol "LDL-c" (mg/dl):

The results in Table (4) showed that mean value of LDL-c in the acute liver disease group "control +ve group increased significantly $p \leq 0.05$, as compared to healthy rats"control –ve group" by about 184.114%.

All treated groups which were suffering from acute liver diseases with the two levels of choline, ZS-C, and their combination showed a significant decrease in the mean value of serum LDL-c, as compared to the positive control group.On the other hand, the mean of serum LDL-c decreased gradually with increasing levels of choline or ZS-C, and or their combination in the prepared diet.

The best results in the mean value of serum LDL-c were recorded for the group fed on a diet containing 2% choline and 7.5% ZS-C leaves, and the group fed on a diet containing 7.5% ZS-C leaves, followed by the group fed on a diet containing 2% choline, respectively.

Serum VLDL-c (mg/dl):

Injecting rats with CCl4 to induce acute liver disease in rats (control +ve group) led to a significant increase in the mean value of serum VLDL-c, as compared to the non-injected group (control –ve group) by about 99.21%.

All treating groups with two levels of choline, or two levels from ZS-C leaves, and or their combinations showed a significant decrease $p \leq 0.05$ in serum VLDL-c, as compared to the positive control group.

The data presented in this table showed non-significant differences in the mean value of serum VLDL-c in all treated groups, except the groups treated with (1% choline and 5% ZS-C leaves), as compared to the negative control group.

In this respect, **(Naik et al., 2012)** found that CCl_4 exposure significantly increased cholesterol, triglycerides, LDL-C, and VLDL-C levels in rats, possibly due to liver and kidney damage.On the other hand,**(Li et al., 2015)** found that CCl_4 exposure significantly increased LDL-C and VLDL-C levels in mice, potentially due to liver damage.

Ziziphusspina-christi aqueous extract demonstrated antioxidant and hypolipidemic activities in hypercholesterolemic male rats, suppressing oxidative stress and enhancing histopathological and biochemical features **(Al-Sieni et al., 2020).** On the other hand, **Irannejad niri et al., (2020)** reported that Dried Z. vulgaris fruit shows anti-hyperglycemic, anti-lipidemic, and anti-inflammatory effects in type 2 diabetes patients.

Sidr Fruit Pulp (SFP) is rich in nutrients like minerals, vitamins, polyphenols, and flavonoids, promoting fat emulsification, hydrolysis, and absorption **(Koo and Noh 2007)**.These effects may explain the antihyperlipidemic effects of SFP.In this respect, **(Kashyap et al, 2019, Dikhanbayeva et al., 2021 and Bencheikh et al., 2021)** reported that Sidr fruits and fermented camel milk have a hypocholesterolemic effect by preventing LDL oxidation.

Choline and betaine supplementation significantly improved lipid profiles in metabolic syndrome patients, lowering total cholesterol, LDL cholesterol, and triglycerides, and increasing HDL cholesterol. On the other hand, **(Wang et al., 2023)** found that choline and betaine supplementation significantly reduced blood pressure and improved lipid profiles in overweight and obese adults. Participants who received choline and betaine supplementation had lower levels of systolic blood pressure, diastolic blood pressure, total cholesterol, LDL cholesterol, and triglycerides, and higher levels of HDL cholesterol, compared to those who did not receive choline and betaine supplementation.

Choi et al. (2020) found that choline supplementation improved lipid profile in overweight and obese adults with Nonalcoholic Fatty Liver NAFLD, decreasing total cholesterol, LDL cholesterol, and triglycerides. It also reduced liver fat, making it a safe and effective treatment option.

Histopathological examination of Kidney:

The kidneys of healthy rats fed on a basal diet revealed the normal histological structure of renal parenchyma (normal renal cortex and renal medulla) (Photos 1 and 2). On the contrary, kidneys of acute liver disease rats fed on a basal diet showed vacuolar degeneration of renal tubular epithelium, focal necrosis of renal tubules associated with inflammatory cell infiltration (Photo 3), and per glomerular inflammatory cell infiltration (Photo 4). On the other hand, kidneys of the acute liver disease group fed on a diet containing 1% choline exhibited congestion of renal blood vessels and glomerular tufts (Photo 5), as well as vacuolar degeneration of some renal tubular epithelium (Photo 6). Otherwise, the kidneys of the acute liver disease group fed on a diet containing 2% choline revealed no histopathological alterations (Photos 7 and 8). Moreover, kidneys of acute liver disease rats fed on diets containing 5% ZS-C leaves showed no histopathological alterations except congestion of renal blood vessels (Photos 9 and 10). Some sections in the kidneys of acute liver disease rats fed on diet containing 7.5% ZS-C leaves described focal necrosis of renal tubules associated with inflammatory cell infiltration (Photo 11), whereas other sections showed no histopathological alterations (Photo 12). Meanwhile, kidneys of acute liver disease rats fed on diet containing 1% choline and 5% ZS-C leaves revealed no histopathological alterations (Photo 13) except vacuolar degeneration of epithelial lining some renal tubules (Photo 14) in some examined sections. Furthermore, the kidneys of

acute liver disease rats fed on diet containing 2% choline and 7.5% ZS-C leaves exhibited no histopathological alterations (Photos 15 and 16).

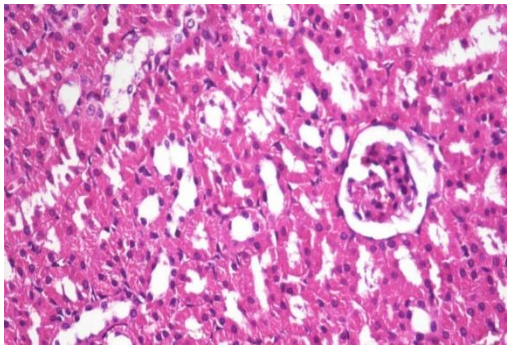

Photo (1): Photomicrograph of the kidney of healthy rats fed on a basal diet showing the normal histological structure of renal parenchyma (H & E X 400).

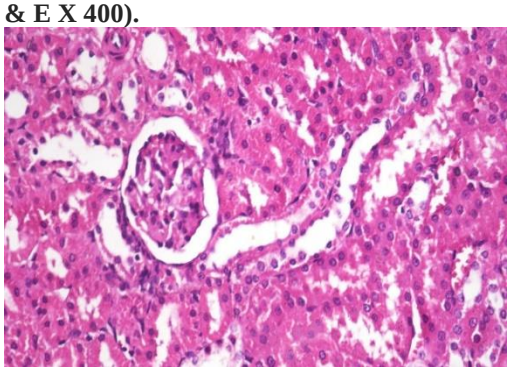

Photo (2): Photomicrograph of the kidney of healthy rats fed on a basal diet showing the normal histological structure of renal parenchyma (H & E X 400).

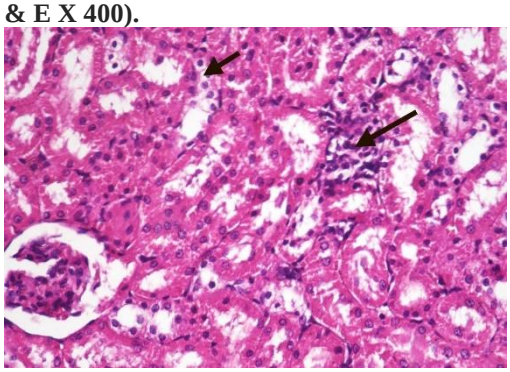

Photo (3): Photomicrograph of kidneys of acute liver disease rats fed on a basal diet showing vacuolar degeneration of renal tubular epithelium (short arrow) and focal necrosis of renal tubules associated with inflammatory cell infiltration (long arrow) (H & E X 400).

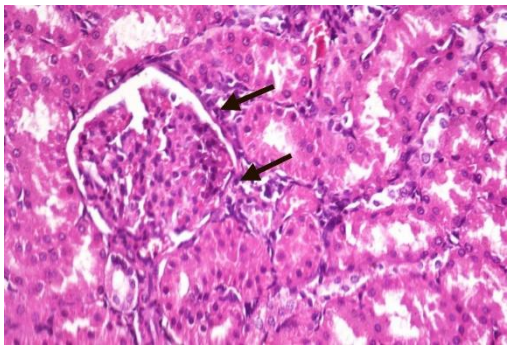

Photo (4): Photomicrograph of kidney of acute liver disease rats fed on a basal diet showing per glomerular inflammatory cell infiltration (arrow) (H & E X 400).

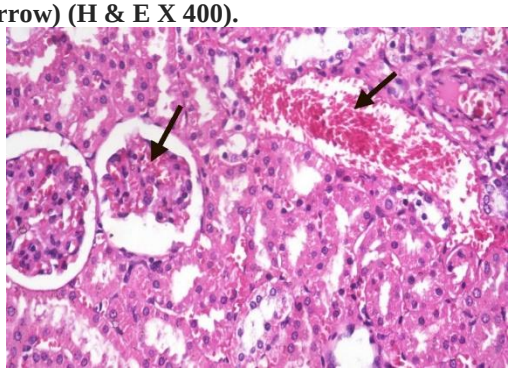

Photo (5): Photomicrograph of kidney of acute liver disease group fed on diet containing 1% choline showing congestion of renal blood vessels and glomerular tufts (arrow) (H & E X 400).

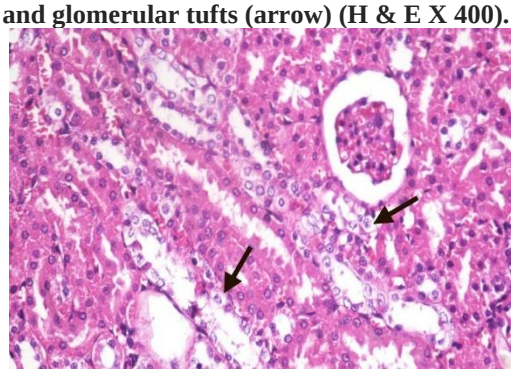

Photo (6): Photomicrograph of kidney of acute liver disease group fed on diet containing 1% choline showing vacuolar degeneration of some renal tubular epithelium (arrow) (H & E X 400).

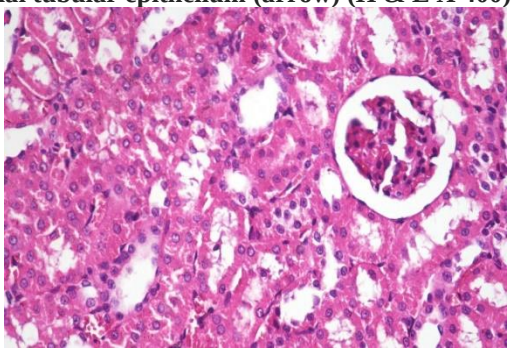

Photo (7): Photomicrograph of kidney of acute liver disease group fed on diet containing 2% choline showing no histopathological alterations (H & E X 400).

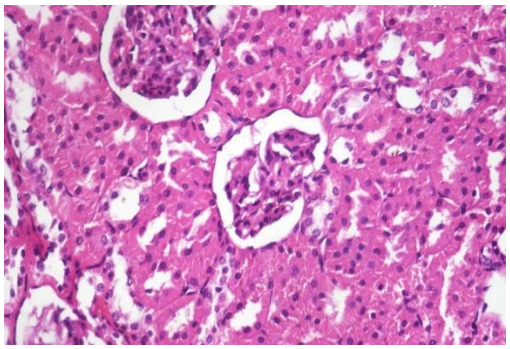

Photo (8): Photomicrograph of kidney of acute liver disease group fed on diet containing 2% choline showing no histopathological alterations (H & E X 400).

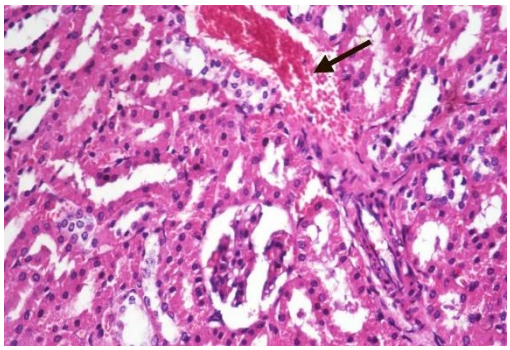

Photo (9): Photomicrograph of kidneys of acute liver disease rats fed on a diet containing 5% ZS-C leaves showing congestion of renal blood vessels (H & E X 400).

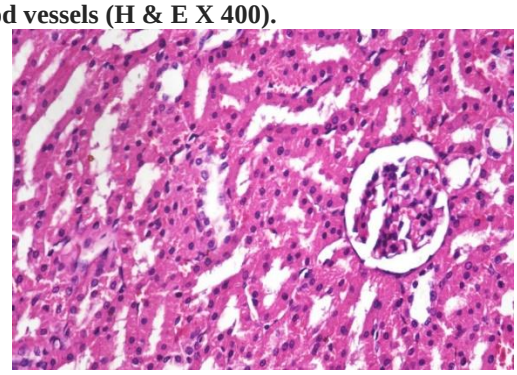

Photo (10): Photomicrograph of kidneys of acute liver disease rats fed on a diet containing 5% ZS-C leaves showing no histopathological alterations (H & E X 400).

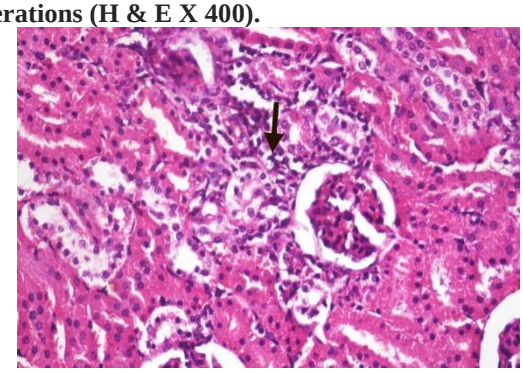

Photo (11): Photomicrograph of kidney of acute liver disease rats fed on diet containing 7.5% ZS-C leaves showing focal necrosis of renal tubules associated with inflammatory cell infiltration (H & E X 400).

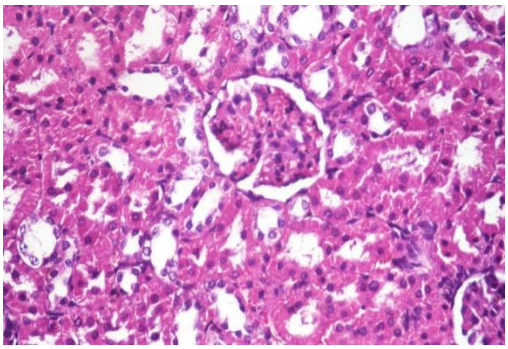

Photo (12): Photomicrograph of kidney of acute liver disease rats fed on diet containing 7.5% ZS-C leaves showing no histopathological alterations (H & E X 400).

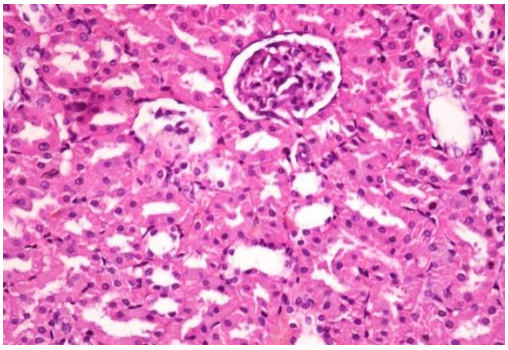

Photo (13): Photomicrograph of kidneys of acute liver disease rats fed on a diet containing 1% choline and 5% ZS-C leaves showing no histopathological alterations (H & E X 400).

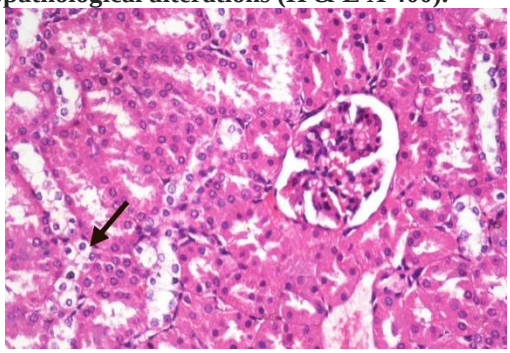

Photo (14): Photomicrograph of kidneys of acute liver disease rats fed on a diet containing 1% choline and 5% ZS-C leaves showing vacuolar degeneration of epithelial lining some renal tubules (H & E X 400).

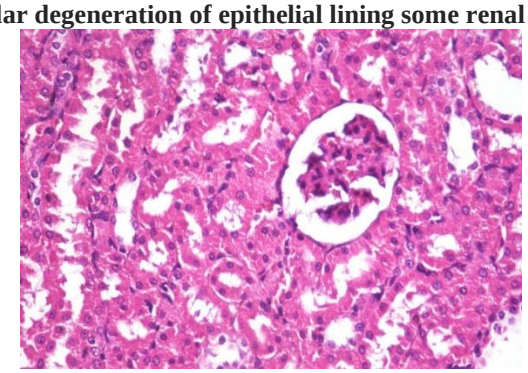

Photo (15): Photomicrograph of kidneys of acute liver disease rats fed on a diet containing 2% choline and 7.5% ZS-C leaves showing no histopathological alterations (H & E X 400).

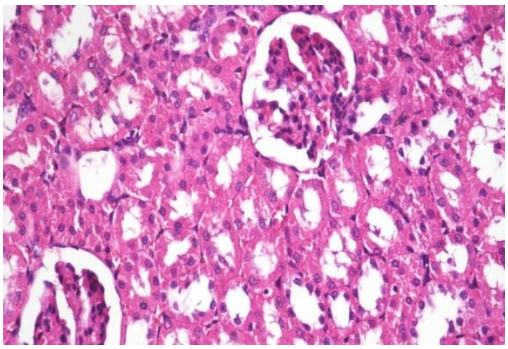

Photo (16): Photomicrograph of kidneys of acute liver disease rats fed on a diet containing 2% choline and 7.5% ZS-C leaves showing no histopathological alterations (H & E X 400).

Conclusion: *Ziziphus Spina-Christi* leaves, choline, and their combinations can be used to enhance the glucose level, uric acid, urea nitrogine, creatinine, HDL-c , LDL-c, VLDL-c, triglycerides and cholesterol in rats induced Acute Liver Disease

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