The antioxidant effect of Jumbolan ethanolic extract seeds against carbon tetra chloride induced kidney toxicity in experimental rats.

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Abstract

Abstract: The present work investigated the effects of *Syzgium cumini L* seeds ethanolic extract against carbon tetrachloride induced kidney toxicity in *Sprague–Dawley* male rats. Twenty four adult male albino rats were divided into two main groups: The first main group (6 rats) fed on basal diet as control negative group (C-ve). The second main group (18 rats) fed on basal diet for 28 days. At 27^{th} and 28^{th} days all rats were administrated ccl4 by gavage (1ml/kgb.w). The second main group was divided into 3 groups each group contained 6 rats as follows: **Group 1**: positive control, rats fed on basal diet only (C+ve). **Group 2**&3: fed on basal diet and received jumbolan seeds extract (250 and 500 mg\kg) orally. At the end of the experiment biological data were calculated, blood samples were taken, kidney collected, weighted. Serum was separated to biochemical analysis. Tissue lipid peroxidation (LPX) and activity of antioxidant enzymes in kidney were also performed. Also histopathological examination for kidneys were performed. Results showed that CCl4 caused elevation in serum levels of creatinine, urea, uric acid, serum potassium, sodium, glucose and liver function. MDA level was also increased significantly whereas SOD, and CAT levels were decreased in the kidney tissue homogenates of CCl4 treated rats. Ethanolic extract of jumbolan seeds successfully improve the alterations of these effects in the experimental animals. Our study demonstrated that the ethanolic extract of jumbolan seeds could protect kidney tissues against CCl4-induced oxidative stress by increasing ant oxidative defense activities.

Key words: jumbolan, kidney, creatinine, uric acid, urea, catalase.

1-INTRODUCTION

Exposure of diverse environment pollutants and xenobiotics such as alcohol, paracetamol, carbon tetrachloride (CCl4), thioacetamide are the major cause of liver disorder, which damage the liver and kidney by producing reactive oxygen species (**Obogwu et *al.*, 2014).**

In years past, carbon tetrachloride was widely used as a dry cleaning solvent until it was recognized as a potent hepatotoxin, nephrotoxin and carcinogen. Today, it is primarily used as an organic solvent **(Kovacic *et al*., 2002).** Administration of CCl4 causes an increase in lipid peroxidation products **(Daniels *et al*., 1995; Abraham et al., 1999 and Donder *et al*., 1999)** and a decrease in the activity of enzymes protecting lipid peroxidation in the kidney **(Dogukan *et al*., 2003**). These detrimental effects of CCl4 have been attributed to conversion of CCl4 to highly toxic trichloromethyl and trichloromethyl peroxyl free radicals by cytochrome P450 enzyme, resulting in cell injury **(Sundari *et al*., 1997; Abraham et al., 1999 and Bahcecioglu *et al*., 1999).**

Carbon tetrachloride (CCl4) is a halo alkane used in lots of industrial and chemical applications as a solvent for oils, fats, lacquers, varnishes and resins, as well as a primary material in the production of organic compounds. CCl4 is also a well-known toxin, which causes tissue damage in human and animals **(Ogeturk *et al*., 2004; Babenko and Shakhova, 2008).**Many studies have demonstrated that CCl4 could increase ceramide content in livers and kidneys **(Ichi, *et al*., 2009).**

Some studies have revealed that natural products، containing antioxidant, protect kidney against lipid peroxidation and impairment in antioxidant status induced by CCl4 **(Khan *et al*., 2009). Jamshid et al., (2020)** found that esveratrol shows a protective effect against nephrotoxicity in CCl4 treated rats by reducing oxidative stress status and modulating the TGF-beta signaling.

S. cumini is colloquially named as‘jamun’ in India, ‘black plum’ in Europe, ‘jambolan’ in Spanish-speaking countries, and it is an evergreen tropical tree belonging to the family Myrtacea **(Abhishek and Vindor**, **2011).** All parts of the tree can be used medicinally and it has a long history of use in traditional medicine **(Baliga *et al*., 2011**). Various traditional practitioners in the Indian subcontinent use the different parts of this tree in the treatment of a wide range of conditions, including diabetes, blisters in the mouth, cancer, colic, diarrhea, digestive complaints, dysentery, piles, pimples and stomachache **(Rizvi and Mishra, 2013).**

The seeds are reported to contain jamboline, traces of pale yellow essential oil, chlorophyll, fat, resin, albumen, tannins **(Jadhav *et al*., 2009)**, phenolic

compounds such as ellagic acid, gallic acid, caffeic and ferulic acids and their derivatives **(Williamson, 2002)** and flavonoids like rutin and quercetin **(Sharma *et al*., 2008).** Health-promoting activities of phenolic compounds present in jambolan reported as anti-inflammatory, anti-allergic, antihyperglycaemic, anticancer, cardioprotective, radioprotective, antibacterial, chemopreventive and antioxidant agents **(Singh et al., 2018).**

Based on such constituents, seed extracts are expected to possess excellent astringent and antioxidant potential, which may be beneficial in relieving gastroenteritis, liver and kidney inflammation. In the present study, CCl4 was used to induce oxidative stress in rats because of its well characterized mechanism, causing structural membranes damage and centrilobular necrosis. Therefore, this study was performed to investigate the protective effects of ethanolic jumbolan seeds extract onCCl4-induced oxidative stress, kidney injury in adult albino rat.

2-MATERIALS AND METHODS

2/1 .Materials:

-Jumbolan fruits were be obtained from a private farm in Tanta governorate.
-Sunflower oil and starch were purchased from the local market.
-Casein, cellulose, vitamins & minerals, dextrin, L-cysteine, choline chloride, and Ccl4 were obtained from the Cairo Company for Chemical Trading, Cairo, Egypt.
-Twenty four male albino rats (*Sprague Dawley strain*) were obtained from the laboratory animal colony, Helwan, Cairo - Egypt. Weighting were approximately between (150-180g).

2/2. Methods:

2/2/1.Preparation of ethanolic seeds extract:

Fruits were washed, after washed in water, the seeds were separated from their pulps and then dark dried for 5 days. The dried samples were ground to fine powder with electric grinder. About 100 g of jumbolan seeds was put in a 1L beaker with 400 ml ethanol (95%), and the mixture was left overnight in the dark then the mixture was heated over mantel with continuous stirring for about 12 hrs. simmered for 10-15 minutes, then left to cool, steep covered for 10-15 minutes, and then the mixture filtered with filter paper. Finally the filtrate was left on the mantel to evaporate the solvent (ethanol) till near dryness.

2/2/2.Chemical composition of jumbolan seeds:

Protein, fat, moisture, ash of seeds determined according to the methods of the **A.O.A.C, (2010).** Total carbohydrates will be calculated as following: Carbohydrates % = 100 - (moisture % + protein % + fat % + ash %).

2/2/3.Determination of antioxidant activity for jumbolan seeds:

Antioxidant activity determined by stable free radical diphenylpicrylhydrazyl (DPPH) Method to estimate the activity of antioxidants according to **Santos *et al* (2013).**

2/2/4. Experimental design:

Twenty four a dult male albino rats, *Sprague Dawley* strain, weighing (150-180g) used in this study were kept in wire cages. The diet was introduced to the rats in special food cups to avoid scattering of food. Also water was provided to the rats. Food and water were provided *ad-libitum* and checked daily. The rats were divided into two main groups: The first main group (6 rats) fed on basal diet as control negative group (C-ve). The second main group (18 rats) fed on basal diet for 26 days. At 27^{th} and 28^{th} all rats were administrated ccl4 by gavage (1ml/kgb.w). This second main group was divided into 3 groups each group contained 6 rats as follows:-

Group 1: positive control, rats fed on basal diet only (C+ve).

Group 2: rats fed on basal diet and received jumbolan seeds extract (250mg/kg).

Group 3: rats fed on basal diet and received jumbolan seeds extract (500mg/kg) orally.

At the end of experiments, all rats were fasted overnight sacrificed and the blood samples were collected, a part of blood was centrifuged to obtain the serum. Internal organs were collected and removed (kidney, cleaned in saline solution, dried by filter paper and weighted. The left kidney was kept in formalin saline 10% for histopathological examination. The right kidney was kept at -80°C for preparation of tissue homogenate for determination of antioxidant parameters. The homogenate was centrifuged at10, 000 rpm for 20 min. The supernatant was used for the assay of some laboratory analyses.

2/2/5.Biological evaluation:

During the experimental period (28 day), the consumed diet was recorded everyday (feed intake), body weight was recorded every week and feed efficiency ratio (FER) **(Chapman et al., 1959)**.

2/2/6.Biochemical analysis:

Albumin, was determined according to **(Drupt, 1974),** total protein **(Sonnenwirth and Jaret, 1980)**. Serum creatinine **(Faulkner and King, 1976**), serum uric acid (**Fossati et al., 1980**) and urea nitrogen **(Patton and Crouch, 1977)**, sodium **Henry (1974)** and potassium **Henry (1964)**. Total iron **Siedel et al., (1984).** ALP according to **Kind and King (1954**), aspartate amino transferase (AST) and alanine amino transferase (ALT) were determined according to **Bergmeyer *et al*. (1986).** Glucose **(Trinder , 1959).**

2/2/7.Assessment of oxidant/antioxidant activity:

Lipid peroxidation by thiobarbuturic acid-reactive substances (TBARS) **(Uchiyama and Mihara 1978),** Superoxide dismutase (SOD) by method developed by **Misra and Fridovich (1972).**Catalase (CAT) by colorimetric assay (**Sinha, 1972**).

2/2/8.Statistical analysis:

Data were presented as means ±SD Statistical analysis of data were tested for significance using a one way analysis of variance (ANOVA) followed by Duncan's multiple range test using computerized SPSS program **(Snedecor and Cochran 1986).** Values were considered significant at $P < 0.05$.

3-RESULTS:

3/1.Chemical composition of the Jumbolan seeds:

Data presented in table (1) showed the averages g of moisture, protein, fat, carbohydrates, fiber and ash per 100g Jumbolan seeds. The results of chemical compositions in seeds revealed that carbohydrates recorded the highest average followed by, fiber, moisture, protein, ash and fat respectively.

Table 1. Proximate composition of the Jumbolan seeds (dry weight) (g\100 g seed)

Constituent	Seeds
Moisture	4.93±0.32 g
Crude Protein	2.74±0.01g
Crude Fiber	7.67±0.12g
Fats/Oils	1.016±0.36g
Ash	2.67±0.12g
Carbohydrates	80.097±0.53

*****Values are means ± standard deviation of three determinations (n=3)

3/2. The antioxidant activity% in jumbolan seeds extract

The results in table (2) showed the average of antioxidant activity % in jumbolan seeds. The results showed that average of antioxidant activity recorded 80.23 %.

Table 2. The average of antioxidant activity% in jumbolan seeds extract

sample	Antioxidant activity
Jumbolan seeds	80.23%

3/3. Effect of jumbolan seeds extract on body weight gain%, feed intake, feed efficiency ratio and relative kidney weight in rats sufferinf from kidney toxicity.

Data presented in table (3) showed the effect of jumbolan seeds extract on body weight gain (BWG), feed intake (FI), feed efficiency ratio (FER) and relative kidney weight against carbon tetra chloride induced kidney toxicity in rats. The results showed that there was significant decrease for weight gain, feed intake and feed efficiency ratio in positive control group compared with negative group, while jumbolan seeds extract (250& 500 mg/kg) groups showed a significant increase in body weight gain, feed intake feed efficiency ratio compared with positive group ($p<0.05$). Data in this table revealed that there was a significant decrease in relative kidney weight for carbon tetra chloride group compared with normal control group. Also jumbolan seeds extract (250& 500) groups showed improvement in relative kidney weight (.737±.05 &.718±.04) respectively compared with positive group ($p<0.05$).

Table 3. The effect of jumbolan seeds extract on BWG %, FI, FER and relative Kidney weight against carbon tetra chloride induced kidney toxicity in rats

Parameters / Groups	BWG %	FI (g/day rat)	FER %	Relative Kidney Weight
Control(-)	24.22+1.3^{a}	18.75+0.38^{a}	0.10+0.009^{a}	.806±.03^{a}
Control(+)	18.98+0.77^{b}	16.71+0.32^{b}	0.06+.003^{b}	.630±.033^{c}
Seeds extract (250)	20.83+0.46^{a}	18.91+0.22^{a}	0.08 +0.002^{a}	.737±.05 ^{ab}
Seeds extract (500)	22.35+0.78^{a}	18.76+.26^{a}	0.09+0.004^{a}	.718±.04^{b}

Values denote arithmetic means ± SD of the mean. Means with different letters (in the same column are significantly at ($p \leq 0.05$) using one way ANOVA test, while those with similar letters are non-significant.

3/4. Effect of jumbolan seeds extract on Kidney functions in rats suffering from kidney toxicity.

Data in table (4), showed that administration of carbon tetra chloride increased the values of creatinine, uric acid and urea significantly as compared to negative control group. while treated groups with (250&500 mg jumbolan seeds extract\kg) showed significant decreases in creatinine, uric acid and urea compared to positive control group. The best result was in jumbolan seeds extract (500mg/kg).

Table 4. The effect of jumbolan seeds extract on serum creatinine, uric acid and urea against carbon tetra chloride induced kidney toxicity in rats

parameters / groups	Creatinine (mg/dl)	Uric acid (mg/dl)	urea (mg/dl)
Control(-)	.286±.04^{c}	2.25±.35^{c}	14.53±1.82^{c}
Control(+)	.625±.10^{a}	5.56±.45^{a}	24.16±1.16^{a}
Seeds extract (250)	.393±.09^{b}	2.36±.33^{c}	17.41±1.68^{b}
Seeds extract (500)	.371±.07^{bc}	2.53±.22^{c}	14.76±2.46^{c}

Values denote arithmetic means ± SD of the mean. Means with different letters (in the same column are significantly at ($p \leq 0.05$) using one way ANOVA test, while those with similar letters are non-significant.

3/5. Effect of jumbolan seeds extract on serum sodium, potassium and iron in rats suffering from kidney toxicity.

Table (5) illustrated the results of serum electrolytes of negative control group, positive control group and treated groups with (jumbolan seeds extract 250& 500 mg/kg). Mean value of sodium and potassium in positive control group were significant increased as compared to negative control group .All treated groups with (jumbolan seeds extract 250& 500 mg/kg) showed significant decreases as compared to positive control group. Total iron recorded a significant decrease in rats administrated carbon tetra chloride compared to normal control group. Whereas, there were a significant increase in treated groups compared to positive control group.

Table 5. The effect of jumbolan seeds extract on serum sodium, Potassium and total iron against carbon tetra chloride induced kidney toxicity in rats

parameters / groups	Sodium (mmol/l)	Potassium (mmol/l)	Total ironµg/dL
Control(-)	144.83±2.99^{b}	6.8±.33^{b}	142.33±0.06^{a}
Control(+)	155.50±3.01^{a}	8.13±.25^{a}	101.83±0.04^{d}
Seeds extract (250)	144.16±.75^{b}	6.10±.77^{b}	122.83±0.02^{c}
Seeds extract (500)	143.50±2.42^{bc}	6.20±.93^{b}	138.17±0.06^{b}

Values denote arithmetic means ± SD of the mean. Means with different letters (in the same column are significantly at ($p \leq 0.05$) using one way ANOVA test, while those with similar letters are non-significant.

3/5. Effect of jumbolan seeds extract on serum protein, albumin and glucose in rats suffering from kidney toxicity.

Table (6) depicts that the serum levels of total protein; albumin were significantly lowered in carbon tetra chloride treated group (+ve) in comparison to control group (-ve).Treated groups significantly increased the levels of serum protein. The results obtained indicated that the CCl4 intoxicated group revealed a high significant increase in the mean value of serum glucose (255.50+22.24 mg/dl) as compared with the normal group (82.05+10.46 mg/dl). The serum glucose of the treated groups were (92.20+3.74 and 78.17+1.94 mg/dl), which were very close to the normal group.

Table 6. The effect of jumbolan seeds extract on serum total protein, albumin and glucose against carbon tetra chloride induced kidney toxicity in rats

parameters / groups	Total protein (g/dl)	Albumin (g/dl)	Glucose(mg /dl)
Control(-)	5.53±.27^{ab}	3.54±.23^{a}	82.05±10.46^{cb}
Control(+)	4.026±.10^{c}	2.96±.16^{b}	255.50±22.24^{a}
Seeds extract (250)	5.59±.27^{a}	3.70±.06^{a}	92.20±3.74^{c}
Seeds extract (500)	5.12±.36^{b}	3.27±.71^{ab}	78.17±1.94^{b}

Values denote arithmetic means ± SD of the mean. Means with different letters (in the same column are significantly at ($p \leq 0.05$) using one way ANOVA test, while those with similar letters are non-significant.

3/6. Effect of jumbolan seeds extract on liver enzymes in rats suffering from kidney toxicity.

Results illustrate that Alanine transaminase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) enzymes activity significantly increased in positive group (+ve) compared with (-ve) control group. While jumbolan seeds extract (250& 500) groups reduced these enzymes activity when compared with (+ve) group, respectively.

Table7. The effect of jumbolan seeds extract on serum (ALT, AST, and ALP) against carbon tetra chloride induced kidney toxicity in rats

parameters / groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Control(-)	48.50±0.1^{a}	22.80±0.10^{a}	146±1.00^{a}
Control(+)	79.50±0.10^{c}	34.50±0.10^{c}	383±1.00^{d}
Seeds extract (250)	53.30±0.10^{b}	29.50±0.10b	263±1.00^{c}
Seeds extract (500)	55.60±0.10^{b}	27.50±0.10^{b}	222±1.00b

Values denote arithmetic means ± SD of the mean. Means with different letters (in the same column are significantly at ($p \leq 0.05$) using one way ANOVA test, while those with similar letters are non-significant.

3/7.Antioxidant SOD, CAT and lipid peroxide (MDA) in kidney tissue:

From (table7) as a result of administering Ccl4,there were a marked reduction in the levels of some antioxidants in the kidney tissue such as catalase (CAT) and superoxide dismutase (SOD), in carbon tetra chloride group (+ve) as compared to negative control group. Also, administration of Ccl4 stimulated lipid peroxidation as estimated by a significant elevation in lipid peroxidation by-product malondialdehyde (MDA). Jumbolan seeds extract (250& 500 mg/kg) reduced the levels of MDA and stimulated antioxidant activity in kidney tissue compared to (+ve) group.

Table 8. The effect of jumbolan seeds extract on antioxidant SOD, CAT and lipid peroxide (MDA) in kidney tissue against carbon tetra chloride induced kidney toxicity in rats

parameters / groups	(nmol\mg) MDA/gm. kidney tissue	(Unit) SOD (u\mg) kidney tissue	CAT (ng\mg) kidney tissue
Control(-)	.082±.01^{a}	.220±.02^{d}	.224±.02^{cd}
Control(+)	.242±.03^{d}	.113±.01^{a}	.075±.01^{a}
Seeds extract (250)	.163±.01^{c}	.127±.02^{b}	.140±.01^{b}
Seeds extract (500)	.167±.02^{c}	.180±.01^{bc}	.167±.01^{c}

Values denote arithmetic means ± SD of the mean. Means with different letters (in the same column are significantly at ($p \leq 0.05$) using one way ANOVA test, while those with similar letters are non-significant.

3/8.Histopathological examination of kidneys:

Samples taken from the kidneys of rats in all groups processed for paraffin embedding. The samples were cut into 4-μm histological sections, which were stained with Masson's trichrome and examined under light microscopy. The light microscopic examination of normal control (-) group kidney sections (photo 1) showed normal orientation of nephrons with adequate glomeruli and well-spaced tubules and no inflammation, no necrosis, no congestion. It is clear that from photo (2) represented that the light microscopic examination of injured group which received carbon tetra chloride at the end of experiment there were characteristic of acute tubular necrosis, tubular cell necrosis; moderate tubular lumen dilation; marked foci of denuded basement membrane; mild intraluminal casts; swelling/flattening of proximal tubular cells with brush border loss; diffuse interstitial edema; interstitial inflammatory cell infiltrates. Photo (3) showed that the light microscopic examination of kidney sections for rats which received jumbolan seeds extract 250 mg /kg^{-1}/ & carbon tetra chloride there were tubular cell necrosis; nil (no) tubular lumen dilation; moderate foci of denuded basement membrane; mild intraluminal casts; nil swelling/flattening of proximal tubular cells with brush border loss; mild diffuse interstitial edema; nil interstitial inflammatory cell infiltrates: mild. Also Photo (4) showed that tubular cell necrosis; mild tubular lumen dilation; mild foci of denuded basement membrane; mild intraluminal casts; nil swelling/flattening of proximal tubular cells with brush border loss; mild diffuse interstitial edema; nil interstitial inflammatory cell infiltrates: mild in rats received jumbolan seeds extract 500 mg /kg^{-1}/ & carbon tetra chloride.

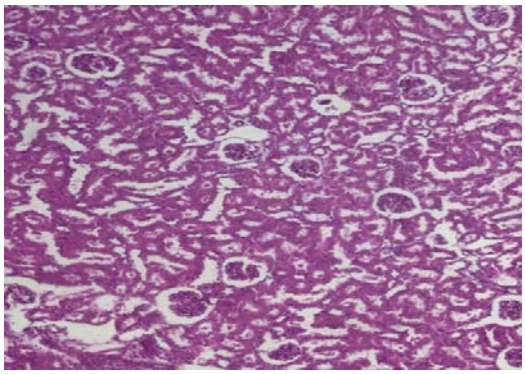
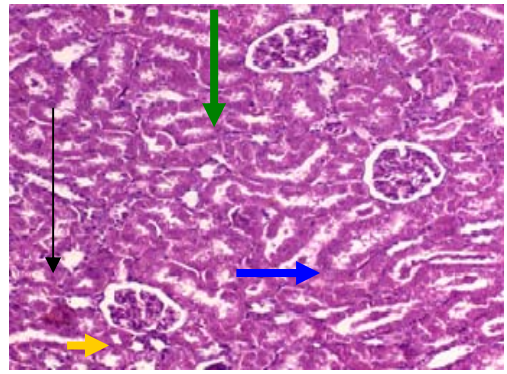


Photo (1): Kidney section from control (-) group of normal rats received basal diet and tab water showed normal orientation of nephrons with adequate glomeruli and well-spaced tubules (H&E,x100).



Photo(4): treated group showed: tubular cell necrosis (blue arrow); tubular lumen dilation (yellow arrow); foci of denuded basement membrane, intraluminal casts (red arrow) ;swelling/flattening of proximal tubular cells with brush border loss (green arrow) and interstitial inflammatory cell infiltrates (black arrow)

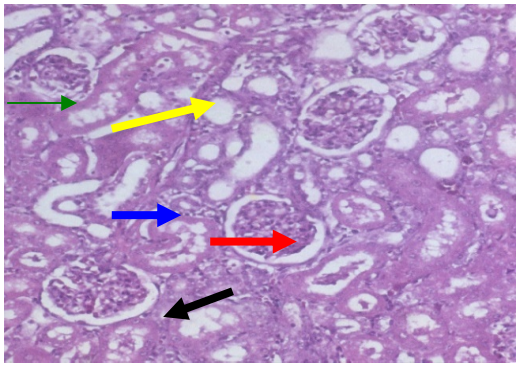
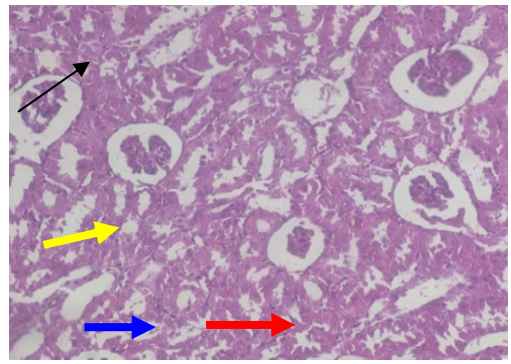


Photo (2): carbon tetra chloride treated group showed: tubular cell necrosis (blue arrow); tubular lumen dilation (yellow arrow); foci of denuded basement membrane; intraluminal casts (short black arrow); swelling/flattening of proximal tubular cells with brush border loss (green arrow); diffuse interstitial edema; and interstitial inflammatory cell infiltrates (black long arrow) (H&E,x100,x200).



Photo(3): treated group showed: tubular cell necrosis (blue arrow); tubular lumen dilation (yellow arrow); foci of denuded basement membrane; swelling/ flattening of proximal tubular cells with brush border loss (green arrow);and interstitial inflammatory cell infiltrates (black arrow) (H&E,x100,x200).

4- DISCUSSION:

In this study, we hypothesized that ethanolic seeds extract of *Syzgium cumini L* would effectively protect kidneys by its antioxidant and anti-inflammatory effects on CCl4-induced injury. The present study demonstrated that ethanolic jumbolan seeds extract inhibited CCl4-induced oxidative stress, inflammation and apoptosis probably through regulating ceramide, MAPKs, calpain and mitochondrial pathway.

Binita et al., (2017) found that seeds are moderately rich in protein (6.3-8.5%) and contains so many other phytochemicals. In the current study, CCl4 induced kidney toxicity in rats and consequently decreased BWG % which also accompanied with decreased FER compared with negative control group. **Lee et al., (2007) and Khan et al., (2012)** reported that injection of Ccl4 was significantly decreased body weight gain and food intake.

Feed intake and body weight gain was significantly increased in rats had received jumbolan seeds extract (250mg/kg) & (500 mg/kg). These may be due to presence of polyphenols which present in jumbolan seeds while improve and protect liver cells against damage via increasing both the levels and activities of antioxidant enzymes in liver and kidneys according to **(Jachec et al., 2002)** leading to improvement the appetite and increasing feed intake. Relative kidney weight decreased in rats treated with CCl4 and increased in rats treated with jumbolan seeds. Mechanism of CCl4-induced damage in kidneys is by causing inflammatory response. Excessive ROS induced by CCl4 could also lead to increased inflammatory cytokines production **(Ma et al., 2015). (Jie-Qiong Maa et al., (2018)** found that mice receiving CCl4 alone showed kidney injury as evidenced by elevation in serum biochemical markers, inflammation, caspase-3 activity and apoptosis in kidney. These changes may occur due to the reduction in the glomerular filtration rate (GFR) or may be secondary due to the oxidative stress, which can cause contraction of mesangial cells, alteration of filtration surface area, and modification of the ultrafiltration coefficient factors that thereby reduces GFR **(Saikat Sen et al., 2018)**.

These data suggests that jumbolan seeds extract was effective in reduction of blood urea, creatinine, and uric acid levels in rats with kidney damage induced by ccl4**. Nahid et al., (2016)** found that jumbolan seeds extract reduced uric acid, creatine phosphokinase, and lactate dehydrogenase. **Ayyanar and Subash-Babu (2012)** documented that jambolan seeds contained phenolic acids, flavonoids and tannins. Jumbolan seeds contain myricetin, quercetin, rutin, ellagic acid and gallic acid have been reported to be effective on kidney injury.

The administration of ccl4 significantly increased the serum sodium and potassium concentration, decreased total iron in rats. Toxic chemicals, certain drugs, infectious agents can induce damage to the kidney that ultimately leads to the imbalance of electrolyte **(Rajakrishnan et al., 2017)**. Also in my study it could be noticed that rats treated with ccl4 had an increase in hepatic enzymes, glucose and a decrease in total protein and albumin when compared with negative control group. Histology of the CCl4 treated group revealed inflammation and damage of liver cells. **Islam et al., (2015)** concluded that methanol extract of seeds of *Syzygiumcumini*, increased serum protein level in CCl4 treated group. **Binita et al., (2017)** mentioned that seeds are rich with phytochemicals. These phytochemicals may provide versatile benefits by influencing biological pathways and improve the diabetic symptoms. Also, liver protective activity of the extract was attributed to saponins, tannins and flavonoids. In the present study, administration of ethanolic extract of seeds of the plant in two doses lowered the level of biochemical markers, which were increased by free radicals of CCl4. It is probable that the administration of extract for 28 days increased the antioxidant capacity of animal to scavenge the free radicals generated by CCl4.

Also my results also showed that CCl4 caused a marked increase in MDA levels whereas SOD and CAT levels were decreased in kidney tissue homogenates of CCl4 treated rats. Ethanolic seeds extract successfully prevented the alterations of these effects in the experimental animals. These findings are in line with those of **Shereen et al., (2015)** and **Rahmouni et al., (2017).** CCl4 when administrated is distributed and deposited to organs such as the liver, brain, kidney, lung and heart**.** The reactive metabolite trichloromethyl radical (•CCl3) and trichloromethyl peroxide radical (CCl3O2•) has been formed from the metabolic conversion of CCl4 by cytochrome P-450. As O2 tension rises, a greater fraction of •CCl3 present in the system reacts very rapidly with O2 and more reactive free radicals, like CCl3OO• is generated from •CCl3. These free radicals initiate the peroxidation of membrane poly unsaturated fatty acids (PUFA), cell necrosis, GSH depletion, membrane damage and loss of antioxidant enzyme activity. Jumbolane seeds were be antitumor and antioxidative **(Goyal et al., 2010)**. **And (Arun et al., 2011).**

Seeds are known to contain ellagic acid, gallic acid and quercetin. Health-promoting activities of phenolic compounds present in jambolan reported as anti-inflammatory, anti-allergic, antihyperglycaemic, anticancer, cardioprotective, radioprotective, antibacterial, chemopreventive and antioxidant agents **(Singh et al., 2018).**

5- CONCLUSSION

Jumbolan seeds have high contents of bioactive phytochemicals, such as phenolic acids, condensed tannins, and flavonoids, including ellagic acid, gallic acid and quercetin which improve liver function and kidney toxicity against carbon tetra chloride.

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الملخص العربى

التأثير المضاد للأكسدة لبذور مستخلص الجامبوزا الإيثانولي ضد رابع كلوريد الكربون الذي يسبب التسمم الكلوي في فئران التجارب

تبحث هذه الدراسة تأثير المستخلص الإيثانولي لبذور الجامبوزا ضد السمية الكلوية التي يسببها رابع كلوريد الكربون في ذكور فئران الألبينو. استخدم في هذه الدراسة عدد ٢٤ فأرا بالغا تم تقسيمهم إلى مجموعتين رئيسيتين : المجموعة الرئيسية الأولى (٦ فئران) تم تغذيتها على غذاء أساسي و استخدمت كمجموعة ضابطة سالبة. المجموعة الأساسية الثانية (١٨ فأرا) تم تغذيتها على غذاء أساسي لمدة ٢٨ يوما ، و في اليوم السابع و العشرون و الثامن و العشرون تم حقن كل فئران هذه المجموعة بمادة رابع كلوريد الكربون (١ مللي|كجم وزن) ويخلط مع البارافين السائل بنسبة (١: ١ ، حجم / حجم) . المجموعة الرئيسية الثانية تم تقسيمها إلى ثلاث مجموعات ، كل مجموعة تحتوي على ٦ فئران كالتالي:- المجموعة الأولى: مجموعة ضابطة إيجابية تم تغذيتها على غذاء أساسي. المجموعة الثانية و الثالثة : تم تغذيتهم على غذاء أساسي مع إعطاء فئران هذه المجموعات (٢٥٠ و ٥٠٠ ملجم مستخلص ميثانولي لبذور الجامبوزا\ كجم وزن) عن طريق الفم.

فى نهاية التجربة تم حساب التقديرات البيولوجية واخذت عينات الدم وفصل الكلى ووزنها ثم اجراء التحاليل البيوكمائية وكذلك الانزيمات المؤكسه مثل MDA والانزيمات المضاد للأكسدة فى نسيج الكلى . كما تم إجراء الفحص التشريحي للكلى.أظهرت النتائج ان رابع كلوريد الكربون تسبب في ارتفاع ملحوظ في مستويات مصل الدم من الكرياتينين واليوريا وحمض البوليك وبوتاسيوم الدم والصوديوم والجلوكوز ووظائف الكبد. كما أحدثت ارتفاعا في مستوى MDA بشكل ملحوظ بينما انخفضت مستويات SOD و CAT في أنسجة الكلى للفئران المعالجة برابع كلوريد الكربون. نجح المستخلص الإيثانولي لبذور الجامبوزا في تحسن أنسجة الكلى . أظهرت الدراسة أن تقليل المستخلص الإيثانولي لبذور الجامبوزا يمكن أن يحمي أنسجة الكلى من الإجهاد التأكسدي الناجم عن CCl4 عن طريق زيادة أنشطة الدفاع ضد الأكسدة.

الكلمات المفتاحية: الجامبوزا – الكلى– الكرياتينين– اليوريك اسيد –اليوريا –الكتاليز.