Hepatoprotective and Antioxidant Activity of Zinnia Elegans Leaves Ethanolic Extract

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Abstract — This study aimed to evaluate the hepatoprotective and antioxidant activity of Zinnia elegans leaves ethanolic extract comparing with silymarine, as standard in rats. The data revealed a highly amounts of phenolic compounds (2.6mg/g d.w of plant), which significantly reflected an antioxidant scavening activity (88%) at 250ppm.The hepatoprotection activity of Zinnia elegans leaves ethanolic extract (50, 100 and 125 mg/100g b.w) comparing with silymarine (0.2 g/kg b.w) against CCl4 toxicity when the Zinnia elegans leaves ethanolic extract improved the AST, ALT and recovered the activity of kidney function by decreasing the urea and creatinine content on the other hand, the administration of Zinnia elegans leaves ethanolic extract significantly suppress the oxidative stress via its direct scavenging against the reactive oxygen species under CCl4 stress. The results reported a decrease in the MDA, H2O2, NO accumulation and increase of GSH content. Finally the administration of Zinnia elegans leaves ethanolic extract have been improved the lipid profiles, LDL and HDL recovered also, the administration of Zinnia elegans leaves ethanolic extract significantly suppress the CCl4 toxicity via its activation of antioxidant enzymes (GST and SOD). The results showed that The Zinnia elegans leaves play an important role in the antioxidant hepatoprotective activity against CCl4 toxicity.

Index Terms— Zinnia elegans, hepatoprotective, antioxidant, scavening activity, oxidative stress and CCl4.

1 INTRODUCTION

The Zinnia elegans (syn. Z. violacea) is native to Mexico and Central America and now has world-wide importance as a garden plant. Tall, mid-sized, and dwarf varieties of this species have been grown for decades, and flowers are available in a wide range of colors. Z. angustifolia (also known as Z. linearis) is less common in gardens, but is gaining in popularity. The plants have narrower foliage and smaller single flowers [1]. Flavonoids are major compounds in flowers and herb of zinnia such as flavonoids, glycosides, tannins, anthocyanins, saponins and phenols [2]. Through the supplement of phytochemicals, Zinnia elegans leaves damage induced may be useful as a hepatoprotective agent indeed retarded the liver injury by blocking the oxidative stress. Therefore, may be useful as a hepatoprotective agent against chemical-induced chronic liver fibrosis in vivo.

Carbon tetrachloride (CC14) is one of the most commonly used hepatotoxins for inducing liver injury in experimental animal studies [3]. Carbon tetrachloride was formerly used for metal degreasing and as a dry-cleaning fluid, fabric spotting fluid, fire-extinguisher fluid, grain fumigant and reaction CCl4 is a potent environmental hepatotoxin has been served as a model compound for study of hepatotoxicity and the cellular mechanisms behind oxidative damage and further was used to evaluate the therapeutic potential of drugs and dietary antioxidants [5]. Liver is prone to xenobiotic-induced injury because of its central role in xenobiotics metabolism, its portal location within the circulation, and its anatomic and physiologic structure [6]. CCl4 is known to induce reactive oxygen formation, and reduce antioxidant enzyme and antioxidant substrates to induce oxidative stress that is an important factor in acute and chronic liver injury. The liver injury induced by CCl4 is resulted from free radicals and lipid peroxidation that cause hepatic cell damage. CCl4 requires bio activation by phase I cytochrome P450 system in liver to from reactive metabolic trichloromethyl radical (CCl•3) and proxy trichloromethyl radical (•OOCCl3).These free radicals can bird with polyunsaturated fatty acid (PUFA) to produce alkoxy (R•) and proxy radicals (ROO•), that, in turn, generate lipid peroxide, cause damage in cell membrane, change enzyme activity and finally induce hepatic injury or nicrosis [7]. Natural products such as flavonoids, terpenoids and steroids etc. have received considerable attention in recent years due to its diverse pharmacological properties including antioxidant activity [8]. This study aimed to prevent liver necrosis through the antioxidant activity of Zinnia elegans leaves ethanolic extract.

2 MATERIALS AND METHODS

2.1 Plant material

Zinnia elegans was collected in April 2012 from pharmacology farm.

2.2 Preparation of ethanolic extract

The leaves of Zinnia elegans were dried in an air oven at 50 ^{o}C till complete dryness. The leaves were ground to fine powder. The dried leaves of Z. elegans were mixed with ethanol (70%) under shaking for 2 days. The resulting ethanolic extract was filtered and subsequently concentrated with a rotary evaporator under low temperature (40 ^{o}C) and reduced pressure.

2.3 Chemicals:

Ferric chloride, Folin-Ciocalteu's reagent, ethanol 2, 2-diphenyl-1-picrylhydrazyl (DPPH), kits reagents. All chemicals and reagents were purchased from Sigma Chemical Co. (London, Lab. Poole), England (Cairo branch). All other chemicals and solvents were analytical grade.

2.4 Phytochemical analysis of Zinnia elegans leaves ethanolic extract

2.4.1 The qualtitative analysis of phytochemical analysis or secondary metabolites such as alkaloides, steroids, saponins, flavonoids, phenols and glycosides. The metabolites were determined according to Harborne methods [9].

2.4.2 The quantitative analyses of flavonoids and phenols. Flavonoids contents have been determined according to Markham method [10]. Total phenols were determined according to colorimetry method [11].

2.5 Antioxidant activity

DPPH radical scavenging activity has been determined of extract according to Burits and Bucar method [12].

2.6 Experimental design

The Sprague Dawley albino male healthy rats were obtained from Research Institute of Ophthalmology; Giza, Egypt has been used in the present work. The animals were weighting (100 – 180g) and kept in an air conditioned animal room for two weeks before the present studies start. The animals were fed on basal diet composed according to Lane Peter and Pearson method [13]. After adaptation, 30 of those male rats were divided into 6 groups each group contained five rats as:

A. G1: Negative control: Fed on the basal diet.
B. G2: Positive control: Fed on the basal diet and injected by (10% CCl4).
C. G3: Fed on the basal diet and injected by (10% CCl4) and treated orally with silymarin (0.2 g/kg b. w).
D. G4: Fed on the basal diet and injected by (10% CCl4) and treated orally with Zinnia elegans extract (50 mg/100g b. w).
E. G5: Fed on the basal diet and injected by (10% CCl4) and treated orally with Zinnia elegans extract (100 mg/100g b. w).
F. G6: Fed on the basal diet and injected by (10% CCl4) and treated orally with Zinnia elegans extract (125 mg/100g b. w).

At the end of the experiment, all the animals were subjected to over-night fasting before being scarified by decapitation. The Blood samples were collected with heparin to obtain serum and plasma that were kept at -20^{o}C, for further analysis enzymatic.

2.7 Chemical analysis

AST and ALT activity were determined according to colorimetric method [14]. Urea and creatinine were determined according to A rapid and precise method [15, 16] respectively. Glutathione reduced and Glutathione-s-transferase were determined according to colorimetric method [17, 18] respectively. HDL-cholesterol and LDL – cholesterol were determined according to colorimetric method [19]. Hydrogen peroxide and Lipid peroxide assay were determined according to colorimetric method [20, 21] respectively. Superoxide dismutase activity and Nitric oxide were determined according to colorimetric method [22, 23] respectively.

2.8 Statistical analysis

All values were expressed as mean ± S.E and statistically analyzed for significance using ANOVA Duncan Test of Assistat program. $P<0.05$ was considered as statistically significant [24].

3. Results and Discussion

3.1 Phytochemical analysis of Zinnia elegans leaves Ethanolic extract.

3.1.1 The qualitative analysis of secondary metabolites in Table (1) Show that Zinnia elegans contain of steroids, saponins, flavonoids, phenols and glycosides. The recorded data were in agreement with Athar [25] who found that saponins, phenolic and flavonoids in Z. elegans. The highly content of phenolic and flavonoids play important role as a natural antioxidant compounds in Z. elegans.

TABLE 1
PHYTOCHEMICAL ANALYSIS OF ZINNIA ELEGANS LEAVES ETHANOLIC EXTRACT.

Phytochemical constituents	Z. elegans +Ve/-Ve
Alkaloids	-Ve
Saponins	++Ve
Phenols	+Ve
Steroids	+Ve
Flavonoids	++Ve
Glycosides	+Ve

+++ High ++ Medium + Low

3.1.2 The quantitative analysis of secondary metabolites such as flavonoids and phenols. The data are shown in Table (2) that Total flavonoids content and total phenols of Zinnia elegans leaves ethanolic extract reflected a highly content of phenols and flavonoids (2.60mg/g and 0.61mg/g) of dry weight respectively. The recorded data were in agreement with Samson [26] who found that the methanolic extract of E.

Alba showed the highest content of phenolic compounds and flavonoids.

TABLE 2

TOTAL AMOUNT OF PLANT PHENOLS AND TOTAL FLAVONOIDS COMPOUNDS OF ZINNIA ELEGANS LEAVES ETHANOLIC EXTRACT.

Plant	Total flavonoids	Total phenols
	mg/g d. w of plant	mg/g d. w of plant
Zinnia elegans leaves	0.61± 0.61	2.60± 0.34

Values are mean if ± SD of three parallel measurements.

3.2 DPPH radical scavenging activity

The data are shown in Table (3) and Fig (1) that The administration of different concentration of Zinnia elegans leaves ethanolic extract (100, 200 and 250 ppm) significantly reflected a highly free radical scaveniging activity (39.83, 77.03, 88.63%) respectively compared to that of silymarine (100ppm) was 40.20%, and this means that Zinnia elegans showed a highly scavenging activity of DPPH radicals, this due to the synergistic effect of antioxidant compound content in the extract. The results are in agreement with Prakash [27] who revealed that the selected plants would exert several beneficial effects by virtue of their antioxidant activity and could be harnessed as drug formulation and reported that Zinnia elegans leaves ethanolic extract significantly inhibited the activity of DPPH radicals.

TABLE 3

.ZINNIA ELEGANS LEAVES ETHANOLIC EXTRACT SCAVENGING ACTIVITY OF DPPH.

Samples	Conc. (ppm)	Scavenging activity (%)
Zinnia elegans leaves	100	39.83±1.21c
	200	77.03±1.09b
	250	88.63±1.04a
Silymarin	100	40.20±0.62e
LSD 5%		11.24

Results are mean ± SD of three parallel measurements.Values with the same letter are not significantly different at P<0.05.

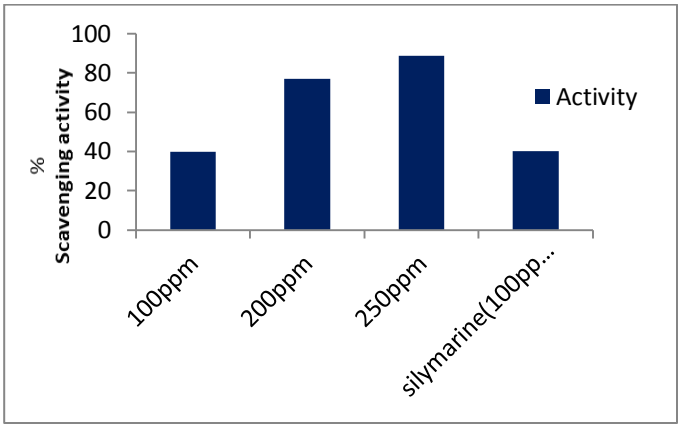

Fig.1. Effects of Different concentrations of Zinnia ***elegans leaves ethanolic*** *extract on scavenging activity of DPPH.*

3.3 Biochemical analysis

3.3.1 Liver function of the experimental animals.

The data are shown in Table (4) and Fig. (2) That the adminstration of different concentration of Zinnia elegans leaves ethanolic extract (50, 100 and 125mg/100g b.w) as well as, standard drug (silymarin) significantly recovered the activity of the AST and ALT enzymes against positive control. The lowest contents have been recorded (21.67 and 28.33 U/L of AST and ALT respectively) with 125 mg/100g b.w of Zinnia elegans leaves ethanolic extract against positive control (118.3 and 114.3 U/L of AST and ALT respectively) these results may be due to the direct antioxidant scavenging activity of the Zinnia elegans extract phenols and flavonoids compounds. These data were in agreement with Murugaian [28] who reported that the improvement of ALT and AST activity with administration of Zinnia elegans extract and it could preserve the normal functional status of the liver.

TABLE. 4.

LIVER FUNCTION OF THE EXPERIMENTAL ANIMALS.

Treatments	AST		ALT	
	U/L	%	U/L	%
Negative control NC	41.67±2.08c	100	35.67±3.05ef	100
Positive control (CCL4)	118.3±2.08a	284	114.3±3.05b	320
Silymarine (0.2g/kg b.w)	32±1de	77	38±2.64cd	107
50mg/100g b.w	36±3.60cd	86	44±0.00c	123
100mg/100g b.w	32±1.73de	77	33.33±2.51fg	93
125mg/100g b.w	21.67±2.88f	52	28.33±4.72g	79
LSD 5% b.w	4.57		5.56	

Each value represents the mean of 5 rats (Mean ± SE).

The same letters in each column represents the insignificant difference at P<0.05.

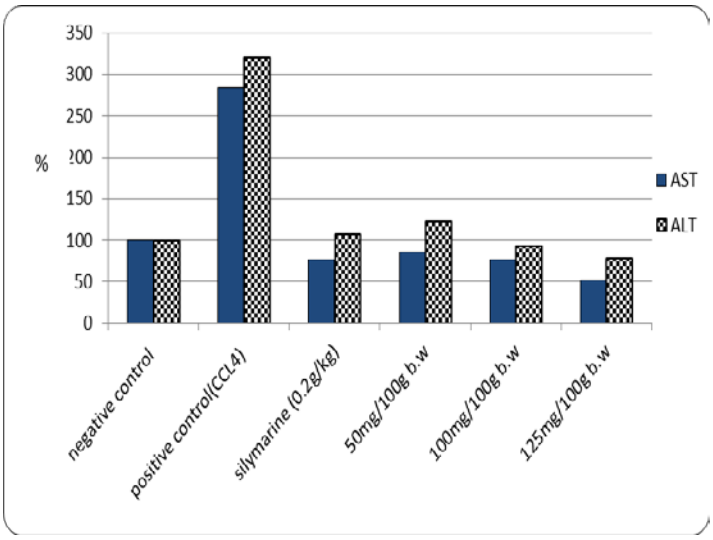

Fig.2.The Effects of** different concentrations of zinnia elegans leaves Ethanolic extract **on AST and ALT activity of experimental CCl4-intoxicated male albino rats

3.3.2 Kidney functions of the experimental animals.

The data are shown in Table (5) and Fig. (3) That the adminstration of different concentration of Zinnia elegans leaves ethanolic extract (50, 100 and 125mg/100g b.w) as well as, standard drug (silymarin) significantly improved of the kidneys function against positive control. The lowest contents have been recorded (28 and 0.6 mg/dl of urea and creatinine respectively) with 125 mg/100g b.w against positive control (62.33 and 1.66 mg/dl of urea and creatinine respectively) these results may be due to the direct antioxidant effects of phenols and flavonoids. These data were in agreement with Abdul jalal [29] who found that a significant improvement of urea and creatinine activity with administration of Zinnia elegans ethanolic extract is due to the presence of phenols and flavonoids which might offer antioxidant activity and recover the kidney function activity.

3.3.3 Lipoprotein profile of the experimental animals

The data are shown in Table (6) and Fig. (4) That the adminstration of different concentration of Zinnia elegans leaves ethanolic extract (50, 100 and 125 mg/100g b.w) as well as, standard drug (silymarin) significantly increased the level of HDL (60 mg/dl with 50 mg/100g) and decreased the level of LDL (83.33 mg/dl with 125 mg/100g of Zinnia elegans leaves ethanolic extract) against positive control (22.67 and 146 mg/dl with HDL and LDL respectively) these results may be due to the direct antioxidant effects of phenols and flavonoids. These data were in agreement with Wang [30] who found that a significant improvement of LDL and HDL content with administration of TNJ juice appears to protect the liver from chronic exogenous CCl4 exposures via phenols and flavonoids compounds.

TABLE 5.
KIDNEYS FUNCTION OF THE EXPERIMENTAL ANIMALS

Treatments	Urea		Creatinine	
	mg/dl	%	mg/dl	%
Negative control NC	25.33±2.30de	100	0. 7±0. 1b	100
Positive control (CCL4)	62.33±5.85a	246	1.66±0.28a	237
Silymarine (0.2g/kg b.w)	30.33±0.57cd	120	0.63±0.05b	90
50mg/100g b.w	30.33±4.04cd	120	0.76±0.15b	109
100mg/100g b.w	32±2.00c	126	0.73±0.23b	104
125mg/100g b.w	28±0.00c	111	0.6±0.00b	86
LSD 5%	3.09		0.26	

Each value represents the mean of 5 rats (Mean ± SE).
The same letters in each column represents the insignificant difference at P<0.05.

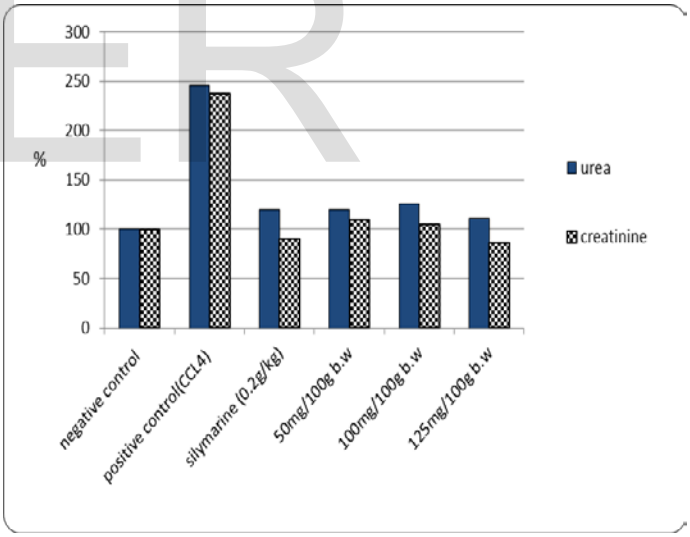

Fig.3.The Effects of** different concentrations of zinnia elegans leaves Ethanolic extract **on urea and creatinine of experimental CCl4-intoxicated male albino rats.

TABLE (6).
LIPOPROTEIN PROFILE OF THE EXPERIMENTAL ANIMALS.

Treatments	HDL		LDL	
	mg/dl	%	mg/dl	%

Negative control NC	90.33±5.50a	100	86.67±4.72e	100
Positive control (CCL4)	22.67±16.19e	25	146±6.92a	168
Silymarine (0.2g/kg b.w)	47.67±2.88cd	53	60.33±1.52g	70
50mg/100g b.w	60±10.00b	66	121.3±2.30c	140
100mg/100g b.w	49.33±2.08cd	55	88.33±2.51e	96
125mg/100g b.w	55±7.00bc	61	83.33±2.51e	96
LSD 5%	12.52		14.47	

Each value represents the mean of 5 rats (Mean ± SE).
The same letters in each column represents the insignificant difference at P<0.05.

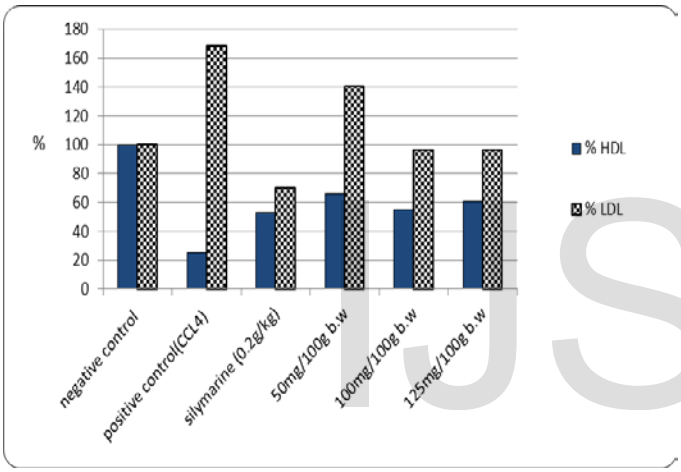

Fig. 4. The Effects of** different concentrations of zinnia elegans leaves Ethanolic extract **on HDL and LDL-cholesterol of experimental CCl4-intoxicated male albino rats.

3.3.4 Liver enzymes such as MDA, GSH as well as the activity of GST and SOD of the experimental animals.

The data are shown in Table (7) and Fig. (5). That the adminstration of different concentration of Zinnia elegans leaves ethanolic extract (50, 100 and 125 mg/100g b.w) as well as, standard drug (silymarin) significantly improved of Superoxide dismutase (SOD), Glutathion-S-Transferase (GST), Glutathione reduced (GSH) and lipid peroxide (MDA) activities. The highest activity of SOD, GST and GSH (618.3, 6.66 U/g and 11.3 mg/g respectively) and the lowest activity of MDA (4.66 nmol/g) with 100 mg/100g of Zinnia elegans leaves ethanolic extract and against positive control (458.3, 3.33 U/g, 3 mg/g and 18.6 nmol/g of SOD, GST, GSH and MDA respectively),The hepatoprotective activity and antioxidant potential of Zinnia elegans ethanolic extract was investigated against CCl4 induced liver damage via its protection activity against the accumulation of MDA under CCl4 stress that induce reactive oxygen formation and reduce antioxidant enzymes and antioxidant substrates to induce oxidative stress. These data were in agreement with Lizby [31] who found that a significant increase of SOD, GST and GSH activities and a significant reduced MDA activity with administration of Zinnia elegans ethanolic extract reduce the accumulation of MDA when suppress the peroxidation of poly unsaturated fatty acids moderated MDA.

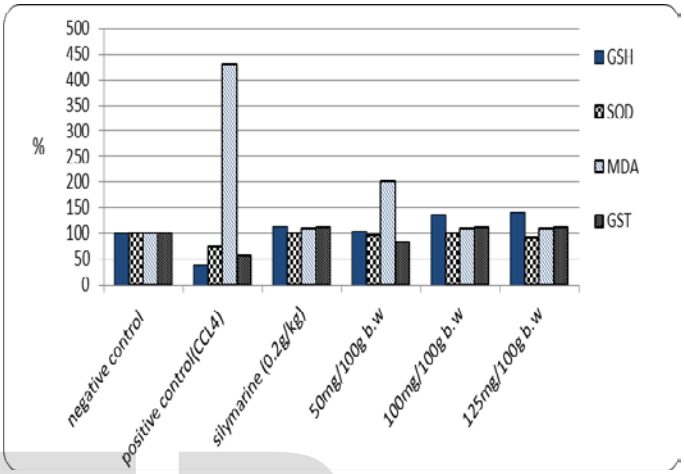

*Fig. 5. The Effects of **different concentrations of zinnia elegans leaves Ethanolic extract** on MDA, SOD, GSH, and GST of experimental CCl4-intoxicated male albino rats.*

3.3.5 Liver oxidation Nitric oxide and Hydrogen peroxide of the experimental animals.

The data are shown in Table (8) and Fig. (6) That the adminstration of different concentration of Zinnia elegans leaves ethanolic extract (50, 100 and 125 mg/100g b.w) as well as, standard drug (silymarin) significantly reduced the NO and H2O2 contents (9.3 and 39.6 µM/L respectively) with 125 mg/100g b.w against positive control (44.0 and 157.3 µM/L of NO and H2O2 respectively) these results may be due to the direct antioxidant effects of phenols and flavonoids. The hepatoprotective activity and antioxidant potential of Zinnia elegans ethanolic extract was investigated against CCl4 induced liver damage via its protection activity against the accumulation of NO and H2O2 under CCl4 stress. These data were in agreement with Bahmanzadeh [32] who found that Nitric oxide and Hydrogen peroxide were increased as a result of oxidative stress and the improvement of NO and H2O2 content with administration of Zinnia elegans ethanolic extract reduce the accumulation of NO and H2O2 under CCl4 stress.

TABLE.7.
LIVER LIPID PEROXIDATION, GLUTATHIONE REDUCED AS WELL AS THE ACTIVITY OF GLUTATHION -S-TRANSFERASE AND SUPEROXIDE DISMUTASE OF THE EXPERIMENTAL ANIMALS.

Treatments	SOD	GST	GSH	MDA

	U/g	%	U/g	%	mg/g	%	nmol/g	%
Negative control NC	623.3±2.88a	100	6.00±0.00b	100	8.00±1.00bc	100	4.33±0.57f	100
Positive control (CCL4)	458.3±2.88d	74	3.33±0.57e	56	3.00±0.00d	38	18.67±1.52b	431
Silymarine (0.2g/kg b.w)	617.7±2.51a	99	6.66±0.57a	111	9.00±0.00b	113	4.66±0.57ef	108
50mg/100g b.w	594±0.00b	95	5.00±0.00cs	83	8.33±0.57bc	104	8.66±1.15c	200
100mg/100g b.w	618.3±2.88a	99	6.66±0.57ab	111	11.30±0.00a	138	4.66±0.57ef	108
125mg/100g b.w	575±19.00c	92	6.66±0.57ab	111	11.33±1.52a	142	4.66±0.57ef	108
LSD 5%	14.80		0.90		1.50		1.75	

Each value represents the mean of 5 rats (Mean ± SE).
The same letters in each column represents the insignificant difference at P<0.05.

TABLE 8.
LIVER OXIDATION; NITRIC OXIDE AND HYDROGEN PEROXIDE OF THE EXPERIMENTAL ANIMALS.

Treatments	NO		H2O2	
	µM/L	%	µM/L	%
Negative control NC	5.66±0.57f	100	27.00±2.64f	100
Positive control (CCL4)	44.0±3.46b	777	157.3±2.30a	583
Silymarine (0.2g/kg b.w)	5.66±0.57f	100	24.33±1.52f	90
50mg/100g b.w	17.33±0.57c	306	77.67±3.51d	288
100mg/100g b.w	11.67±1.52d	206	78.33±7.57d	290
125mg/100g b.w	9.33±0.57de	165	39.67±5.13e	147
LSD 5%	3.10		8.25	

Each value represents the mean of 5 rats (Mean ± SE).
The same letters in each column represents the insignificant difference at P<0.05.

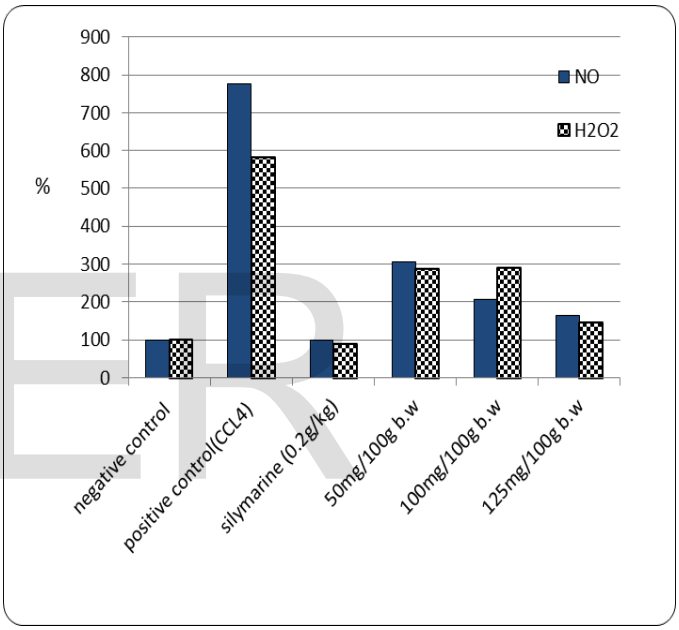

Fig. 6.The Effects of *different concentrations of zinnia elegans leaves Ethanolic extract* ***on*** *H2O2 and NO levels* ***of experimental CCl4-intoxicated male albino rats.***

4. CONCLUSION

In the present study, the effect of Zinnia elegans leaves was evaluated in vitro and in vivo as therapy in experimental CCl4-intoxicated albino rats. The results revealed a high content of total phenols, significantly increase of DPPH, improvement of AST, ALT, NO, H2O2, LDL, MDA, urea, creatinine, GST, SOD and HDL by Zinnia elegans leaves extract. The results showed that the Zinnia elgans leaves have a hepatoprotective activities against CCl4 induced toixicity on rats. Zinnia elegans leaves damage induced may be useful as a hepatoprotective agent indeed retarded the liver injury by blocking the oxidative stress.

5. REFERENCES

[1] Fanelli FL, Dole JM, Fonteno WC, Harden BT. Optimizing Postharvest Life of Cut Zinnias. Department of Horti. Culture. Sci., North Carolina State University, Raleigh 2014; 2: 7695-7609.

[2] Yamaguchi M, Terahara N, Shizkuishi K. Acetaylatedanthocyanins in Zinnia elegansflowers. Phytochemistry, 1990; 20 (4): 2269-2270.

[3] Temitope MO, Damilola SA, Chukwuemelie ZU. Antioxidant capacity of Moringa oleifera seed oil against CCl4-induced hepatocellular lipid peroxidation in wistar albino rats. Euro J Exp. Bio. 2014; 4(1): 514-518

[4] DeShon ND. Carbon Tetrachloride. Kirk-Othmer Encyclopedia of Chemical Technology. Vol. 5, (3rded), John Wiley and Sons, New York, 1979, pp. 704 – 714.

[5] Basu S. Carbon tetrachloride-induced lipid peroxidation: eicosanoid formation and their regulation by antioxidant nutrients. Toxicology, 2003; 189(1-2): 113–127.

[6] Jones A, Dolak Robert L, Waller D, Eric A, Glende Jr, Recknagel Richard O. Liver Cell Calcium Homeostasis in Carbon Tetrachloride Liver Cell Injury. J Biochem. Toxicology, 2006; 3(4): 329-342.

[7] Weber WD, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. Critical reviews in toxicology, 2003; 33(2): 105-136.

[8] Chen YR, Zweier JL. Cardiac mitochondria and reactive oxygen species generation. Circulation Research, 2014; 114: 524–537

[9] Harborne JB. "Phytochemical Methods", 2nd edn. Chapman and Hall, London, 1984

[10] Markham KR. "Techniques of Flavonoid Identification ". Academic Press, London, 1982, Vol. 2, pp. 427-468.

[11] Singleton VL, Rossi JAJ. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Viticult, 1965; 16: 144-158.

[12] Burits M, Bucar F. Antioxidant activity of Nigella sativaessential oil. Phytotheraphy Res., 2000; 14: 323–328.

[13] Lane Peter WA, Pearson AE. Dietary requirements.In:The laboratory animal principles and practice. Acedemic press Eds. London, New York, 1971, PP. 142

[14] Reitman S, Frankel SA colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Anal J Clin. Path, 1957; 28: 56.

[15] Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. J Clin. Pathol, 1960; 13: 156-159.

[16] Schirmeister J, Willmann H, Kiefer H. Plasma creatinine as rough indicator of renal function. Dtsch Med Wochenschr, 1964; 89: 1018-1023.

[17] Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin. Med, 1963 ; 61: 882-888.

[18] Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first step in Mercapturic acid. J Biol Chem, 1974; 249: 7130-7139.

[19] Wieland H, Seidel DA. simple specific method for precipitation of low density lipoproteins. J Lipid Res, 1983; 24(7): 904-909.

[20] Ortega E, Marcos S, Galban J. Fluorometric enzymatic autoindicating biosensor for H2O2 determination based on modified catalase. Biosensors & bioelectronics, 2013; 41: 150-156.

[21] Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta, 1978; 90(1): 37-43.

[22] Nishikimi M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun, 1972; 46: 849-854.

[23] Montgomery HAC, Dymock JE. The determination of nitrite in water. Analyst. J Biol Chem, 1961; 86: 414-416.

[24] Silva FAS. The ASSISTAT Software: statistical assistance. In: International Conference on Computers in Agriculture, 6, Cancun, Anais. Cancun: American Society of Agricultural Engineers, 1996, pp. 294-298.

[25] Athar A. Studies on saponin from some indigenous plants. Park Vet J, 1990; 10: 146-148.

[26] Samson Guenne, Nabere Ouattara, Adama Hilou, Jeanne FM, Odile GN. Antioxidant, Enzyme Inhibition Activities and Polyphenol Contents of Three Asteraceae Species used in Burkina Faso Traditionally Medicine. Int J Pharm Sci, 2011; (3)5: 524-528.

[27] Prakash Veeru, Mishra Pankaj Kishor, Mishra Meenakshi. Screening of medicinal plant extracts for antioxidant activity. J Medi Plants Res, 2009, 3(8) pp. 608-612.

[28] Murugaian P, Ramamurthy V, Karmegam N. Hepatoprotective Activity of Wedelia calendulacea L. against Acute Hepatotoxicity in Rats. Res J Agriculture and Biological Sci, 2008; 4(6): 685-87.

[29] Abdul jalal A, Selvakumar S, Nallathambi R, Jeevaprakash G, Dheivanai S, Senthilvelan S. Hepatoprotective Activity of Wedelia Chinensis Against Carbon Tetrachloride Induced Liver Damage in Rats. Int j phytopharmacology. 2012; 3(2): 121-125.

[30] Wang MY, Anderson G, Nowicki D, Jensen J. Hepatic protection by noni fruit juice against CCl(4)-induced chronic liver damage in female SD rats. Plant Foods Hum Nutr, 2008; 63(3): 141-145.

[31] Lizby AM, Dhanyaraj D, Prathibhakumari PV, Prasad G. Hepatoprotective and Antioxidant Potential of Sphaeranthus Indicus [Linn] on Liver Damage in Wistar Rats. Int J Pharm Sci, 2012; 4(3): 222-225.

[32] Bahmanzadeh M, Abolhassani F, Amidi F, Ejtemaiemehr SH, Salehi M, Abbasi M. The effects of nitric oxide synthase inhibitor (L-NAME) on epididymal sperm count, motility, and morphology in varicocelized rat. DARU, 2008; 16(1) 23-28.