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Effect of Cuminum cyinum on some physiological and antioxidant parameters in male rabbits and antioxidant parameters in vitro

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Abstract:

The aim of this study is to explain the effect of Cuminum cyinum (Cc) on some physiological and antioxidant parameters in male rabbits and antioxidant parameters in vitro . Eighteen adult male rabbits were used in this study and divided equally in to three groups , first group was control (C) intubated orally distilled water for four week , the second group treated group (T_1) was intubated orally with(C c) in dose 125 mg ⁄ kg B.W for four weeks , third group received C c in dose 250 mg ⁄ kg B.W (T_2) group blood samples were collected from starved animals at the end of experiment by heart puncture from each animal blood serum was separated from coagulant blood by centrifugation of 5000 rpm for 15 minute separated serum was stored at – 20 C^0 and used for biochemical analysis .preliminary chemical detection of C c of the active compounds it contains alkaloids , saponin , terpenoids and flavonoids . The effect of *Cuminum cyminum (Cc)* on Antioxidant parameters explained that glutathione GSH um / L there was significant increase $p<0.05$ in glutathione concentration in T_1 and T_2 as compared with control . There was non-significant increase $p>0.05$ in Vit.C concentration in T_1 with significant decrease in Vit. C in T_2 $p< 0.05$ as compared with control while there was non-significant increase or decrease in the vit. E concentration $p>0.05$ there was non-significant increase or decrease $p>0.05$ in the oxidative stress marker Malondialdehyde (MDA) concentration $p > 0.05$ um / L in T_1 , T_2 group as compared with control . Lipid profile that include total cholesterol TC there was non-significant $p > 0.05$ increase or decrease in the concentration of total cholesterol , and high density lipoprotein cholesterol HDL- C , VLDL – C and blood sugar while there was significant increase $p < 0.05$ in low density lipoprotein cholesterol concentration LDL- C in T_2 group as compared with control and T_1. kidney function parameters that include urea , Creatinine and uric acid . There was non-significant increase or decrease in the level of urea and uric acid in T_1 and T_2 as compared with control while there was significant decrease $p < 0.05$ in the level of Creatinine in T_2 group $p < 0.05$ as compared with control.

The analysis of liver enzymes that include aspartate transaminase (AST) there was significant decrease $p < 0.05$ in the level of AST , and non-significant increase in the serum alanine aminotransferase (ALT) . While the measurement of serum total protein , albumin and globulin there was non-significant increase or decrease in the concentration of total protein , albumin and globulin in T_1 and T_2 groups as compared with control .The antioxidant capacity in vitro of cumin measured by DPPH assay it had obvious as antioxidant effect.

Key word : Cumin cyminum , Glutathione , MDA , Lipid profile , liver enzymes

INTRODUCTION

Cumin [*Cuminum cyminum*] belong to the family apiaceae is small annual herbaceous plant that is member of aromatic plant [1].its a multipurpose plant that cultivated in Middle east and several Mediterranean countries[2]. Its fruit known as cumin seed that's generally used as food additive to give the flavor and most widely used for traditionally and medicinal purpose that has many pharmacological activities that include: anti-inflammatory [3],analgesic [4],Antimicrobial activity [5] Antiestrogenic [6], antidiabetic [7],Antitussive [8],anti-blood platelet aggregation[9],Antioxidanti [10] Hepatoprotective [11], Anticancer [12],Antistress [13] Antifungal [14],Antiulcer [15].and many other pharmacological effect.cumin is an excellent source of minerals like iron, copper, calcium [16] ,potassium ,manganese, selenium, magnesium. The magnesium of cumin serves a host of function including : promoting heart health, controlling pressure,treatment of leprosy, inflammation, enlarge of spleen, ulcers, corneal opacities also used in Alzheimers, vomiting, cold, anticancer, antitussive, [17], cumin seeds has been found to posses essential oils such as :cuminaldehyde [4-isoproppyl benzaldehyde], pyrazines, 2-methoxy-3 methyipyrazin. 2ethoxy-3isopropyl pyrazine and 2-methoxy-3 methlpyrazine [18]. Its also contain very good amount of B-complex vitamins such as thiamin,vitamin B-6, niacin, riboflavin, and vital anti-oxidant vitamins like [E,A,C].the seed also rich source of many flavonoid phenolic anti-oxidant such as :carotenes, zeaxanthin, and lutein [7].

MATERIAL AND METHODS

Cuminum cyminum seeds after grinding the plant its used in the experiment .

The experiment was conducted at the animal house of biology department , college of science for women/Baghdad University.Eighteen adult male rabbits weighting 1000-1300 gm were used in this study . The animals were housed for two weeks for adaptation , they were kept under good hygienic conditions .animals were maintained on natural 12 h light and 12 h dark cycle , received abalanced diet water ad libitum through out the experimental period, rabbits were divided into three groups (n=6) and treated for four weeks as follows : control group (C) received distilled water orally dialy for four weeks and treated group (T1)received orally *Cuminum cyminumn* in a dose that was 125 mg/kg BW and the animals received this dose orally and dialy for four weeks

Second treated group (T2) received orally *Cuminum cyminum*in a dose that was 250 mg/kg B.w dialy for four weeks . at the end of the experimental period ,overnight fasting blood samples were collected by heart puncture serum was separated from coagulant blood by centrifugation at 5000 rpm for 10 minutes and serum stored at -20 centigrate for studying the following parametters

The antioxidant parameters that include GSH according to [19], Vit C according to [20] . Vit E according to [21] And Malondialdehye (MDA) according to (22)

Determination of serum total cholesterol (TC) concentration using enzymatic assay kit [23], triacylglycerol TAG According to [24] high density lipoprotein cholesterol (HDL-C) concentration according to [25], low density lipoprotein cholesterol (LDL-C) concentration and very low density lipoprotein cholesterol (VLDL-C) concentration according to [26].

kidney function parameter (Urea, creatinine and Uric acid) according to diamond enzyme kit [27 , 28] respectively . liver enzymes (AST , ALT) according to [29] . Serum total protein was estimated as described by [30 , 31] determination of serum albumin concentration g/dl was estimated according to [32] .determination of serum globulin concentration g/dl was estimated indirectly the result of serum globulin concentration g/dl =total serum protein - serum albumin concentration .

Plant material *Cuminum cyminum Cc* seeds was used for the Preparation of the crude The ethanolic *extract*, was prepared following the process described (koppula 2011)[33]; 100 g of *C.c*

seeds . It was separately macerated with the solvents and allowed to stand for 72 hrs and then filtered. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored in labeled sterile screw capped bottles at 5°C in the refrigerator, until when required for use.

Phytochemical screening of *Cuminum cyminum Linn* Chemical tests for the screening and identification of bioactive chemical constituents in the Cress C.c. seeds were carried with extracts prepared using the standard procedures.

Flavonoids [34] 0.5 g of ethanolic was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. The filtrate was used for the following tests: 5 ml of the dilute ammonia solution was added to the portion of the aqueous filtrate of each plant extract followed by the addition of concentrated H2SO4. The appearance of the yellow coloration indicated the presence of flavonoids.

Using Lieberman reagent [35] allows identifying pink- purple ring indicates the presence of terpenes.

Tannins according to [36] Search for catechin tannins is made from reagent Stiasny. 5 ml of the extract were evaporated to dryness. After adding 15 ml of reagent Stiasny the residue, the mixture was kept in a water bath at 80°C for 30 min. The observation of a precipitate in large flakes characterized accelerator tannins. For tannins, we filtered the previous solution. The filtrate was collected and saturated with sodium acetate. The addition of Fecl3drops causes the appearance of a blue-black coloration intense, indicating the presence of tannins.

Alkaloids according to [37] Alkaloids were characterized from Bouchardat reagent (reagent iodo-iodized) and Dragendorff (reagent iodobismuthate of potassium). 6 ml of each solution were evaporated to dryness. The residue is taken up in 6 ml alcohol at 60 °. The addition of 2 drops of reagent Dragendorff on the alcoholic solution caused a precipitate or orange color. Adding 2 drops of Bouchardat reagent on the alcoholic solution caused a color precipitate reddish brown and indicated a positive reaction.

Saponosides according to [38] To find saponins, we contributed in a test tube, 10 ml aqueous total extract. The tube was shaken for 15 s and allowed to stand for 15 min. A height of persistent foam greater than 1 cm indicated the presence of saponins.

Determination of the antioxidant activity invitro Trapping free radical by DPPH assay was conducted to evaluate the antioxidant activity. This test measures the ability of eliminating free radicals in Methanolic extract. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant that can donate an electron to the DPPH, the purple color, typical free radical DPPH to disintegrate, the mixture was kept in the dark for 30 minutes and (OD) was recorded at 517 nm against a control sample [39]. 0.5 ml of Methanolic extract of the test solutions at different concentrations (25,50,100,150,200 ppm) were mixed with 2.5ml of a methanolic solution of 0.1 mM DPPH. The absorbance is measured by UV / VIS device Spectrometer Lambda 25. The percentage inhibition of the DPPH is calculated by the formula as follows [40].

The standard calibration curve is α-tocopherol (Vitamin E).

% I = (Ac - As) / As

Where Ac = absorbance of control and As = absorbance of sample.

Statistical Analysis

The Statistical Analysis System was used to compare the effects of (HS) treated group parameters with control. Least significant difference –LSD test was used to compare significance between means in this study [41].

RESULTS AND DISCUSSION

Table 1 : The effect of *Cuminum cyminum (Cc)* in a dose 125 mg/kg Bw (T1) and 250 mg/kg bw on antioxidant parameters that include Glutathione(GSH) , Vitamin C , Vitamin E and Lipid peroxidation indicator Malondialdehye (MDA) in control T1 and T2 in male rabbits

Mean±				
parameter / Group	**GSH *um/l***	**VIT C mg/l**	**VIT E mg/l**	**MDA *um/l***
Control	3.20 ± 0.07 B	0.76 ± 0.02 a	0.45 ± 0.03 a	0.76 ± 0.01 A
T1 125mg/kg	4.31 ± 0.12 A	0.75 ± 0.04 a	0.54 ± 0.03 a	0.73 ± 0.02 A
T2 250mg/kg	4.20 ± 0.07 A	0.42 ± 0.02 b	0.31 ± 0.04 a	0.72 ± 0.00 A
LSD Value	0.655*	0.186*	0.267 NS	0.208 NS
*(P< 0.05), NS: Non –significant .				

Table 2 : The effect of *Cuminum cyminum (Cc)* on lipid profile on serum Total cholesterol , HDL-C , LDL-C , VLDL-C and blood sugar

Means ±					
Group	**Total cholesterol TC mg/dl**	**HDL_C mg/dl**	**LDL_C mg/dl**	**VLDL mg/dl**	**Blood sugar [BS]**
Control	82.04±3.82 A	20.01± 1.27 a	47.80±2.74 b	13.83± 0.88 b	88.1± 3.62 a
T1	74± 3.75 A	16.23± 2.63 a	45.82± 2.63 b	13.30± 0.88 b	93.00±2.75 a
T2	88.52± 9 A	17.00± 0.67 a	54.50± 3.02 a	17± 0.61 a	90.00±2.86 a
LSD	17.942 NS	5.02 NS	5.96 *	2.6 *	11.629 NS
*p<0.05; NS non-significant ,					

It is appeared that seeds contain polyphenols , flavonoids , and tannin that exhibited antioxidant activities both in vitro and invivo. pharmacological activities [42].

our results explained that there was significant increase $p<0.05$ in glutathione in T1 and T2group as compared with control this explained that Cc exhibited high antioxidant activity due to that the Cc has anti DPPH in our study against free radicals activity that contain flavonoids and other polyphenolic compounds the antioxidant activity has been underlying its effect as anticarcinogen ,antimutagenc and antistress [44 , 42]

Valves expressed as mean ± SE N=6 each group Control group; treatment groups: T1 group and T2 group.

table 4 explained that effect on lipid profile there was non-significant increase in serum total cholesterol and HDL-C in T_1 and T_2 as compared with control with non-significant change in blood sugar there was non-significant increase in LDL-C and VLDL-C in T_1 while there was significant increase in LDL-C and VLDL-C in T_2 as compared with control but there was non-significant decrease in blood sugar in our study there was dis agree with [45] [46] and agree with [47]

In our results there was non-significant increase or decrease in total cholesterol TC and HDL-C . while there was significant increase in LDL-C in T_2 as compared with control and T_1

and also cumin seed powder in 0.5 g/kg with the diet of rabbits showed increase in serum cholesterol in blood . while cumin seed powder 0.25g/kg for 6 weeks to aloxan induced diabetic rats showed significant reduction in plasma and tissue cholesterol , phospholipids free fatty acids and triglyceride .[45 , 46]

there was non-significant increase and decrease in the level of urea and uric acid in T_1 , and T_2 as compared with control group while there was significant decrease in the level of creatinine in T_2 as compared with control our result do not agree with [45]

That explained the effect of C.c lowered blood urea level and reduced the concentration urea and creatinine in diabetic rat [48] while there was significant decrease in the level of creatinine T_2 as compared with control

Table 3 :Explain the effect of C.c. in the kidney function parameters in male rabbits that received 125 mg/kg B.W. (T1) and received 250 mg/kg B.W. (T2) as compared with control

Parameters / Group	Mean ±		
	Urea mg/kg	**Creatinine Mg/dl**	**Uric Acid Mg/dl**
Control	30.12±2.63 a	0.53±0.006 a	4.05±0.15 a
T1 125 mg/kg	31.00±1.91 a	0.57±0.017 a	3.90±0.08 a
T2 250 mg/kg	31.00±1.72 a	0.39±0.003 b	3.51±0.11 a
LSD value	3.722 NS	0.192 *	0.783 NS
*P<0.05 , NS : Non-significant			

Table 4 : The effect of *Cminum cyminum* (C.C) on liver enzymes AST ,ALT and ALP control,T1 that receiving 125 mg/kg of C , and T2 received 250 mg/kgB.W,as compared with control that receive D.W orally for four weeks.

Parameter / Group	Mean± SE	
	AST u/ml	**ALT u/ml**
Control	16.40±0.72 a	19.61±1.06 A
T1 125mg/kg	13.52±0.63 b	18.10±0.93 A
T2 250mg/kg	9.01±0.55c	18.00±1.17 A
LSD value	*	3.892 NS
*(p<0.05),NS:Non-significant		

Table 5 : The effect of *Cuminum cyminum (Cc)* in a dose 125 mg/kg Bw (T1) and 250 mg/kg bw on total protein , albumin and globulin as compare with control group in male rabbits

Mean ±SE			
Parameter / Group	**Total protein T.B mg/dl**	**Albmin mg/dl**	**Globulin mg/dl**
Control	4.78 ±0.35 A	3.09 ± 0.06 a	1.69 ± 0.03 a
T1 125 mg/kg	4.53 ± 0.18 A	3.90 ± 0.11 a	1.36 ± 0.03 a
T2 250 mg/kg	4.72 ± 0.03 A	3.00 ± 0.06 a	1.72 ± 0.05 a
LSD VALUE	1.249 NS	1.096 NS	0.796 NS
*p<0.05 ,NS:Non significant			

Table 6 : The preliminary chemical detection for Cumin Cyminum seeds

Alkaloids	+
Saponin	+
Terpines	+
Tanins	+
Flavonoids	+

Table 7 : Effect of concentration in percentage in anti-oxidation of extraction of cuminum cyminum and ascorbic asid –DPPH

Concentration µg / ml	**Percentage of anti-oxidant**		**LSD value**
	Extraction	**Ascorbic Asid**	
25	15.77±0.72	65.30±2.63	2.922*
50	27.87±1.59	83.60±3.14	3.629*
100	53.78±2.46	98.03±3.71	3.772*
150	100±0.00	98.20±3.62	4.912*
200	100±0.00	98.60±4.68	3.305*
LSD value	5.283*	6.042*	----
*(P<0.05)			

The anti-oxidant capacity of cumin by DPPH assay was 15.77 ± 0.72 , 27.87±1.59 , 53.78±2.46 and 100±0.00 in the concentration of C.c. that was 25,50,100,150, and 200 Mg /ml respectively

Explained that the effect of CC on liver enzymes there was significant reduction in serum AST enzymes in T_1 and T_2 as compared with control that explained its effect as hepato protective I agree with non-significant increase or decrease in the level ALT enzyme [47 , 49] explained that C.c. have a protective effect on liver function study in this result .

there was non-significant increase or decrease in the level of total protein , albumin and globulin concentration in T_1 and T_2 as compared with control as antioxidant modulate carcinogen metabolism decrease the incidence induced hepatoma .the cancer chemopreventive potential of cumin seed could be attributed to its ability to modulate carcinogen metabolism . and there was nonsignificant increase or decrease in the levels of plasma proteins [50 , 51].

Phytochemical analysis According to the percentage of Yield, the highest yield was observed in methanol extract (34.2%) followed by ethyl acetate extract (19.1%) and finally the petroleum ether extract (14.2%). Phytochemical constituents in the plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer. [52]. All secondary metabolite components displayed antioxidant and antimicrobial properties through different biological mechanisms. Most of the secondary metabolite components were isolated and identified in the polar plant crude extracts [53]. The main chemical groups identified in petroleum ether, Ethyl acetate and methanolic crude extracts of the *Lepidium sativum* seeds are mentioned in the Table 6.

The result of the detection of the active compounds it contains alkaloids , saponin , terpenoids and flavonoids .The preliminary chemical test explained that chemical detection of the active compounds in alcoholic extract of C.c.

Table 6: Phytochemical screening of the extract of *Lepidium sativum* seeds Phytochemical screening of the methanolic, petroleum ether and ethyl acetate extracts of *LS* seed revealed the presence of various medically active constituents. The phytochemical compounds present in the methanolic extract were identified as alkaloids, saponins, sterols, tannins, flavonoids and terpenoids (Table-6). Saponins and alkaloid were also present in ethyl acetate and petroleum ether.

The presence of these chemical constituents in the seeds of *L.S* demonstrates to their antibacterial activity. These phytochemicals are known to show medicinal as well as physiological activity [54]. The occurrence of alkaloids, saponins, sterols, tannins, flavonoids [54 , 55]

Antioxidant activity The antioxidant activity through free radical scavenging activity (DPPH) method of different extracts of *LS* was determined. The principle of antioxidant activity is their interaction to produce oxidative free radicals. The role of DPPH method is that the antioxidants react with the stable free radical. During the free radical reaction, DPPH (a,a-diphenyl-b-picrylhydrazyl) is converted into a,a-diphenyl-b-picrylhydrazine with color change. The rate of color change gradually decreases to indicate the scavenging potentials of the sample antioxidant. The IC50 values of the Methanol extracts of Cc was was 100 µg / ml where as the Vit - C 25 µg / ml . The Methanol extracts of *Cc* contain flavonoid, saponins, tannins and alkaloid . All these bioactive compounds were able to discolor DPPH solution by their hydrogen donating ability [53 , 55]. From the results it appears that the Methanol extracts of *Cc* possess hydrogen donating capabilities and it will act as an antioxidant.

The scavenging activity might be due to the presence of total polyphenolic compounds. These polyphenolic compounds include flavonoids, anthraquinones, anthocyanidins, xanthones and tannins . These compounds have been reported to scavenge free radicals, superoxide and hydroxyl radical by single electron transfer [56].

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