American Journal of Ethnomedicine, 2015, Vol. 2, No. 3
Available online at http://www.ajethno.com

ISSN: 2348-9502

Biochemical Effect of Nepeta Septemcrenata Growing in South Sinai, Egypt

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ABSTRACT

The present study aims to at investigate the effect of ethanolic extract of *Nepetaseptemcrenata* on lipid peroxidation and antioxidant defenses, the activities of three lysosmal acid hydrolases and the total protein, DNA and RNA contaent and Hepatic and kidney functions. In this study, three main tests were investigated including; Pharmachological effect of the plant extraction, Biochemical effect of the ethanolic extract and Effect of *Nepetaseptemcrenata* ethanolic extract on liver lysosomal enzymes, total protein, DNA and RNA. The results reviewed that antipyretic effect is the decreasing of the temperature of the hyperthermic rats, theethanolic extract of *Nepetaseptemcenata* which given interperotential to rats with inflamed paw in a dose of (1/5 LD_{50} & 1/10 LD_{50}) significantly decreased the thickness of the inflamed rats' paw as compared with the control group and standard group after 6 hours. The ethanolic extract of *Nepetaseptemcrenata* in a dose of 1.1 and 50.5 mg/kg, induced protection against writhing response andreaches its maximum effect in a dose of 1.1 mg/kg. In theAntibacterial activity the investigation of antimicrobial activities of *Nepetaseptemcrenata*extract revealed that it has no activity against *S. aureus* and a very weak antiseptic action against *E.* coli. Finally, *Nepetaseptemcrenata* hasmuch medicinal prosperity.

Keywords- *Nepetaseptemcenata*, Antioxidant, Egypt.

INTRODUCTION

Nepeta is one of the most important genera of lamiaceae family with regard to the number of its species. Some species of this genus are important medicinal plants and their extracts have been used for medicinal purposes[1]. Some species of *Nepeta* germs are utilized in folk medicine for treatment of contusions, rheumatic pains, fever, cutaneous eruption and some species are used for their anti-inflammatory properties[2]. *Nepeta* plants were prepared as tea and used

in traditional medicine as anthelmintics, febrifuges, expectorants and to treat bronchitis, bites as well as stings of scorpions[3].

Nepetaseptemcrenata is an erect slender plant with branches at base leaves are oppositely alternated, ovate with crenate or slightly dentate margins. This plant is found in Saint Catherine, Sinai, Egypt[4]. *Nepetaseptemcrenata* known to be used by the native Bedouins in folk medicine as antipyretic, sedative, cardiotonic, eye wash and as a gargle in sore throat[5]. An isopimarance type diterpence and 7-omethyl apigenin were isolated from the ethanolic extract of *Nepetaseptemcernata* herb[6,5] investigated the chemical constituents of *Nepetaseptemcrenata* and [7]analyzed the volatile oil of the same plant species[8]. Reported that the lactone- free hot water extract of catnip (*Nepetacatrina*) causes a significant decrease in the wakefulness and an increase in sleep behavior, particularly deep sleep. A few members of genus *Nepeta*are reported to posses biological activities especially reduction of serum lipids and inflammatory effects[6].

*Nepeta*is used in traditional medicine as laxative; to treat dysentery, kidney and liver diseases and teeth troubles[9]. Two methods for the preparation of an oral liquid used in traditional Chinese medicine called Jingfang that contains *Nepetacataria* species[10,11,2] found that *Nepeta*has anti-inflammatory and analgesic activities respectively.[2] Reported the use of *Nepeta* in folk medicine for treatment of contusions, rheumatic pains, fever, and coetaneous eruptions. The main objects of this present study is:1-Biochemical trail to evaluate the effect of the ethanolic extract of the plant on: Lipid peroxidation and antioxidant defenses, Hepatic and kidney functions and the activities of three lysosmal acid hydrolases and the total protein, DNA and RNA content.

MATERIAL AND METHODS

Pharmacological effect of the plant extraction

This experiment was undertaken to determine the analgesic, antipyretic and anti-inflammatory activities of the ethanolic extract of *Nepetaseptemecrenata*. Mice were used to measure the analgesic activity, whereas rats were selected to measure the antipyretic and anti-inflammatory activities of the extract. Animals of these experiments were exposed to the ethanolic extract in doses equal to 101 mg/kg (1/10 LD_{50}) & 50.5 mg/kg (1/20 LD_{50}) for mice and 101 mg/kg (1/5 LD_{50}) & 50.5 mg/kg (1/20 LD_{50}) &50.5 mg/kg (1/10 LD_{50}) for rats. Thus several test activities have been done upon those animals: Analgesic Activity (Acetic acid induced writhing test), antipyretic activity, anti-inflammatory activity and antimicrobial activity.

Biochemical effect of the ethanolic extract

Sample of blood were collected using the orbital sinus technique (Sanford, 1954) after 2, 4, 8, 16 and 24 hours of treatments for determining the following parameters: Lipid peroxidation as malondialdehyde content in the blood plasma, Glutathione content in blood, Catalase activity in blood, Cu/Zn-SOD activity in blood, Plasma Total antioxidant capacity, Liver function tests: ALT& AST and Gamma GT activities in plasma and Kidney function tests: Creatinine and Urea content in plasma. The method used for estimation of Cu/Zn superoxide dismutase activity in blood was described by [12]and [13].

Effect of *Nepetaseptemcrenata* ethanolic extract on liver lysosomal enzymes, total protein, DNA and RNA.

First preparation of liver lysosomal fraction, in which the livers of all animals were immediately removed after sacrificing, whole liver samples were perfused in suit

with 0.025 M in ice-cold sucrose buffer (pH 7.4) and then homogenized for 10 minutes by electric homogenizer and centrifuged at 2500 rpm in a Beckman refrigerating ultracentrifuge (Model J2-21) for 15 minutes[14,15]. Secondly, preparation of the total lysosomal enzymes in which the solutions were freezed and thawed for three successive times to rupture the lysosmal membranes; centrifugation was done at 19.000 rpm for 20 minutes at 4°C. The supernatant was separated in aliquota and kept at -20°C for determination of lysosomal Acid phosphtase (ACP), β-galctosida (GAL) and β-N-acetyl-D-glucosaminidase (β-NAG) enzyme activities. Third, determination of lysosomal acid hydrolyses activities in which the activities of ACP, β-GAL and β-NAG were carried out according to the method described by[16]. Fourth, the fraction and extraction of intercellular components protein, DNA and RNA of liver cells, the modified procedure of[17]was employed as following: (a) Lipid extraction and (b) Protein extraction (c) RNA extraction (d) DNA extraction. Fifth, determination of total proteins was carried out by the method of [18]. Sixth, quantitative determination of RNA as the RNA content was measured colourimetrically by the modified orcinol procedure. Seventh, quantitative determination of DNA, the DNA content was measured following the diphenylamine procedure described by[19].

Statistical analysis

According to the mathematical principles described by Field (2000) all the data of control and treated groups for both(1/5 LD_{50}& 1/10 LD_{50}) were expresses as mean value± standard error. Student unpaired t- test was used to explain variation between control group and each treated group (each time of treatment) in all parameters. Also the results were analyzed by One-way analysis of (ANOVA test) followed by Tukey-Krameis[20].

RESULTS

Acute toxicity and LD50:

After interperitoneal administration of ethanolic extract of *Nepetaseptemcrenata* in mice, (Up-down method) LD_{50} was found to be 1009.58 mg/kg body weight for mice and 504.79 mg/kg for rats.

Pharmacological screening of *Nepetas-etemcrenata*

(a) The antipyretic effect of *Nepeta-septemcrenata*

Antipyretic effect is the decreasing of the temperature of the hyperthermic rats. The results of the antipyretic effects of the tested ethanolic extract (1/5 and 1/10 of *Nepetaseptemcrenata* were shown in tables (1 and 2). From the tables it was concluded that alcoholic extract of *N. septemcrenta* when given interaperiotenal to the included hyperthermeric rats in both, does produced the antipyretic effect as shown by decrease in body temperature, when compared to negative and positive control.

(b) The anti-inflammatory effect of *Nepeta-septemecrenata*

The results of the anti-inflammatory effect of the plant extract are shown in table (3). From this table it's evident that the ethanolic extract of *Nepetaseptemcenata* which was given interperotential to rats with inflamed paw in a dose of (1/5 LD_{50}& 1/10 LD_{50}) significantly decreased the thickness of the inflamed rats' paw as compared with the control group and standard group after6 hours.

(c) The analgestic Effect of *Nepeta-septemcrenata*

The results of analgesic effect are shown in table (4). From the table we can conclude that, the ethanolic extract of *Nepetaseptemcrenata* in a dose of 101 and 50.5 mg/kg b.wt. induced protection against

writhing response, which reach its maximum effect in a dose of 1.1 mg/kg.

Antibacterial activity

Investigation of antimicrobial activities of *Nepetaseptemcrenata* extract revealed that it has no activity against *S. aureus* and a very weak antiseptic action against *E.* coli. The results of antibacterial effect are shown in table (5), which shows that *Nepeta* extract has no activity against *S.aureus*and very weak antiseptic action against *E. coli* at 100 mg/ml.

D-Biochemical study

I-lipid peroxidation

The effect of interperitoneal injection of ethanolic extract of *N. septemcrenata*on lipid peroxidation content (µmol MDA/L) of rat is presented in table (6) and illustrated graphically.

The results indicated that, in the first treated dose ($1/5LD_{50}$) there were significant decreases in plasma lipid peroxidation content with percent of changes equal to 26.03% and 26.85% with respect to the control group after 4 and 8 hours respectively, wheres, non-significant decreases were recorded after 2, 16, and 24 hours. One way- ANOVA between the control and the five treated group (2,4, 8, 16 and 24 hours) revealed a highly significant difference ($F_{1,4}$=5.299, p< 0.001) in lipid peroxidation content. In the second treated dose ($1/10\ LD_{50}$), significant decreases were noticed in plasma lipid peroxidation content with percent of changes equal to 31.78% and 27.67% with respect to the control group after 8 and 24 hours respectively. However, non-significant decreases were recorded after 2, 4 and 16 hours respectively. In addition, a highly significant difference in lipid peroxidation content was observed between the control and the five treated groups (2, 4, 16 and 24 hours) using one-way ANOVA ($F_{1,4}$=6.052, P< 0.0001). The data also showed significant differences between the two treated doses at the time interval of 4 and 24 hours. Blood glutathione (GSH) content, the effect of interpretational injection of ethanolic extract of *N.septemcrenata* on blood glutathione (GSH) content (mg/dl) of rat is presented in table (7). The results indicated that the first treated dose ($1/5LD_{50}$) showed significant increases in blood glutathione content with percent of changes equal to 86.52% and 21.92% when compared with the control group after 2 and 16 hours of treatment respectively. The results showed also non-significant increase in this group after 4, 8 and 24 hours of treatment. Highly significant differences in blood glutathione content were observed between the control and the five treated groups (2, 4, 8, 16 and 24 hours) using one-way ANOVA (f_{14}= 314.44, p<0.001). Blood Catalase activity, the effect of interpretational injection of ethanolic extract of *N.septemcrenata* on blood catalase activity (µ/ml) of rat is presented in table (8). Blood Cu/Zn Superoxide dismutase (SOD) activity, the effect of interpretational injection of ethanolic extract of *N. septemcrenata* on blood superoxide dismutase activity (µ/ml blood) of rat is presented in table (9). Plasma total antioxidant Capacity (TAC), the effect of interpretational injection of ethanolic extract of *N. septemcrenata* on plasma total antioxidant capacity (percentage) of rat is presented in table (10).

DISCUSSION

In recent decades, interest in natural products has been restored by discovering penicillin, plant- derived tranquilizers, and plant precursors of cortisone. Contrary to previous beliefs, botanical drugs are proving more economical than synthetics, and hold forth encouraging prospects of inhibiting or destroying tumor without undue damage to

healthy tissue. Extensive plant screening programs are being conducted by governmental agencies and pharmaceutical companies. Folk remedies, still common in many tropical areas, are being evaluated. No ways the general public is being made aware that herbal drugs are not obsolete but offer new hope for conquering disease[21].

Medicinal plants (also known as herbs, herbal medicine, pharmacologically active plants and phytomedicinaks) are the dominant form of medicine in most countries. More than three-fourth of the current population depend on raw products to meet daily health care needs[22]. Approximately 25% of the drugs available in the average American pharmacy come from plants, either as purified extracts or as partially modified secondary products[23].

Intraperitoneal LD_{50} of alcoholic *Nepetaseptemcrenata* extract was estimated and it was found to be 1009.58 mg/kg body weight for mice and 504, 79 mg/kg body weight for rats. This results in agreement with [5]who reported that the alcoholic extract of *N.septemcrenata* have a wide marginal safety where it's LD_{50} was 421.8 mg/kg body weight in rat. Also this value was a bit close to the LC59 of *Nepetacataria* recorded by [24]. They mentioned that intraperitoneal LD_{50} of *Nepetacataria* oil, nepetalactone-enriched fraction and nepetalic acid were found in mice to be 1300 mg/kg, 1550 mg/kg and 1050 mg/kg, respectively.

[1]Estimated the chemical components of the aerial parts of *Nepeta* sintenisii. The plant subjected to hydrolysis and the chemical composition of the isolated essential oil was analyzed by GC/MS method for the first time. Forty constituents (96.5% of the total oil) were identified. 3neepetalactone (23.4%), elemo (16.1%), E-3-famesene (9.5%), 1.8-cineole (8.2%), cis-sabinene hydrate (6.5%), 3-bisabolene (4.2%) and germacrene-D(3.5%) were the main components.

Importance of *Nepeta*species as medicinal herb is due to its contents of flavinoides. Different classes of flavonoides were previously extracted and identified from many species of genus *Nepeta*[9].The isolation and identification of various flavonols in*Nepetanepetalla* were reported by [25]. The identification of rosmarinic, ferulic, p-coumarric and p-hydroxybenzoic acids in the leaves and flowers of *Nepetanepetella*and *N. tuberose*, and caffeic acid in *N. nepetella*, is described by [26]. In addition[27] isolated and identified the phenolic acids from the stems of *Nepetanepetella* subspecies cordifolia (Willk) and *Nepetatuberose* subspecies reticulate. The stem contains fewer phenolic acids than the leaves and flowers. The following compounds were identified, caffeic, syringic, 4-hydroxybenzoic (p-hydroxybenzoic acid), vanillic, cis-p-coumaric and trans-p-coumaric acids.

Both external and lipophilic aglycones as well as vascular glycosides of the air dried aerial part of *N. transcauasica* were studied [5]stated that, the compounds were identified as: crisimaritin (L VIII), xanthomicrol (LIX), salvigenin (LX), gardenin-B(LXL), apigenin and genkwanin (LXII), and as external aglycones while luteolin, apigenin (LXIII), 7-glucouronides (LXIV), nepetin and hispidulin-glucosided (LXLL), nepetin and hispidulin-7-glucosided (LXV) and 7-glucouroindes (LXVI) as avascular glycoside.

[2]Reported the results of phytochemical studies on aerial parts of *Nepetasibthorpii*, an endemic plant of Greek, the bioassay- guided fractionation of methanol extract led to the isolation of ursolic acid and polyphenol fraction by HPLC. They determined some phenolics: chlorogenic acid (0.315 mg/g) and the flavonoids rutin (0.09mg/g), luteolin-7-o-glucoside (0.387 mg/g) and a luteoin derivative. They assayed the radical scavenging activity of *Nepetasibthorpii*

methanol extract by the 1, 1-dipheny 1-2-picrylhydrazyl (DPPH).

It was found that apigenin as flavonoid of *Nepetaseptemcrenata* extract inhibits the induction of β- galactosidase. Apignin demonstrated potent anti-inflammatory activity in carrageenan included rat paw edema and it was concluded that flavonoids offer important therapeutic potential for the treatment of a variety of inflammatory diseases[28]. It was investigated that the bioflavonoids on lysosmal acid hydrolylases, viz., β-N-acetyl glucosaminidase and cathepsinn D in serum, liver, kidney and spleen and the stability of liver lysosomes was studied by[29]. The activity of these enzymes in arthritic tissue and serum increased significantly. The total activity of β-glucurandase in the lysosome-rich fraction from arthritic liver was appreciably decreased, while its release was significantly increased. It was found that one of the most potent flavones, apigenin as a flanonoid exhibited a dose-and time-depentent, reversible effect on adhesion protein expression as well as inhibitory adhesion protein up regulating at the transcriptional level0[28]. Also, apigenin inhibited 1L-1α-induced prostaglandin synthesis and TNF-a- induced 1L-6 and 1L-8 productions, suggesting that the hydroxyl flavones may acts as general inhibitors of cytokine-induced gene expression. As well as, this falconoid inhibit TNF-α induced β-glactosiddase activity in SW 480 cells stably transected will a β-glactosiddase.

It was found that the natural extract of some medicinal plants protect free radicals damage[30]. In addition, it has been shown that the content of the mucosal non protein. Sulfhydrxyl group may increase by the effect of the active ingredients of the medicinal plants[31]. It was found that protective effects of four flavonoids "quercetin, rutin, luteolin and apigenin" against induced DNA damage in human leukemia cells (K562). These flavonodis are characterized by the number of hydroxyl groups on the B ring, the presence of an O-dihydroxy structure on the B-ring confers a higher degree of stability to the flavonoid phenoxyl radicals by participating in electron delocalization and is, therefore, an important determinant for antioxidant potential[32]. The strongest inhibition against DNA damage (44%, 42%) was found in a range of luteolin and quercetin concentrations of 20-100 u mol/L. It has been investigated that DNA protective capacity of three flavonoids, apigenin, luteolin and quercetin against the free radical generated by H_2O_2. The quantitative analysis has shown that luteolin possesses the highest DNA protective effect of flavonoids[33].

Also, thirteen isoflavonoids, flavonoids, and lignanshave been found to be evaluated for their effects on DNA synthesis in MCF-7 and human breast cancer cells by [34]. At 0.1-10 µM of coumestrol, genistein, biochaninA, luteolin, apigeninkaempferol and enterolactone induced DNA synthesis 150-235% and at 20-90 Um, inhibited DNA synthesis at high concentrations but induction at concentration close to probable levels in humans. [35]Investigated that the flavonoides "qurestin, rhamnetin, isorhamnetin, apigenin, and luteolin" did not introduce any damage into the DNA.

In conclusion, *N. septemcrenata* has many promising active constituents that exert significant pharmacological and therapeutic effects with high margin of safety. We recommend further phytochemical and pharmacological studies on each purified active constituent of this plant on different body systems and on fatal diseases such as cancer and AIDS.

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Table 1: Antipyretic effect of ethanolic extract of *Nepetaseptemcrenata* (1/5 LD_{50}) in rats

Treatments	Temperature						
	Before injection	Fever	After 30 min	After 60 min	After 120 min	After 180 min	After 240 min
Yeast extract	36.5±0.071	37.16±0.154	37.22±0.16	37.32±0.195	37.36±0.204	37.3±0.232	37.24±0.22
Antipyretic drug(Aspegic)	36.64±.051	38.02±0.32*	37.68±0.28	37.46±0.273	37.04±0.133	36.76±0.103	36.5±0.123
Plant ethanolic extract(1/5 LD_{50})	36.5±0.141	36.6±0.141	36.6±0.192	36.28±.211*	36.26±0.258**	36.46±0.24*	36.7±0.259

Values are mean ± S.E. (n=5, *= p < 0.05(significant), **= P 0.01 (highly significant)

Table 2: Antipyretic effect of extra of *Nepetaseptemcrenata* (1/10 LD_{50}) in rat

Treatments	Temperature						
	Before injection	Fever	After 30 min	After 60 min	After 120 min	After 180 min	After 240 min
Yeast extract	36.5±0.071	37.16±0.154	37.32±0.159	37.32±0.195	37.36±0.204	37.3±0.232	37.24±0.216
Antipyretic drug(Aspegic)	36.64±0.051	38.02±0.318*	37.68±0.281	37.46±0.273	37.04±0.133	36.76±0.103	36.5*±0.123
Plant ethanolic extract(1/5 LD_{50})	36.5±0.126	37.5±0.217	37.26±0.196	36.26±0.221*	36.34±0.225**	36.82±0.168*	37.24±0.140

Values are mean ± S.E. (n=5), *= P < 0.05(significant), **= P 0.01 (highly significant)

Table 3: Anti-inflammatory effect ethanolic extract of *Nepetaseptemcrenata* (1/5 LD_{50}) & (1/10LD_{50})

Group	Thickness of paw in mm after (hrs)			
	Before yeast	4h. after yeast	3h.after treatment	6h.after treatment
Control	4.26±0.098	8.46±0.582	8.64±o,0.520	8.62±o.548
Inflammatory Drug (Diclofenac Sodium)	4.22±.0735	8.82±0.269	6.66±0.309**	6.6**±0.342
Plant Ethanolic Extract (1/5 LD_{50})	4.09±.0678	8.875±0.401	7.48±0.22	7.08±0.146*
Control	4.26±0.098	8.46±0.582	8.64±o,0.520	8.62±o.548
Inflammatory Drug (Diclofenac Sodium)	4.22±.0735	8.82±0.269	6.66±0.309**	6.6**±0.342
Plant ethanolic extract (1/10 LD_{50})	4.09±.0678	8.875±0.401	7.48±0.22	7.08±0.146*

Values are Mean±S.E (n=5)& (n=10) *= p<.05 (signification) **= p<.01 (highly significant)

Table 4: Analgesic activity of ethanolic extract of*Nepetaseptemcrenata* in mice using writhing technique

Treatment	Writhing response(No/20 min)
Control	21.2±5.4
Plant ethanolic extract(1/10 LD_{10})	2.4±0.678**
Plant ethanolic extract(1/20LD_{50})	8.2±1.068**

Values are mean ± S.E (n=10) *= p<.05 (significant) **= p<.01 (highly significant)

Table 5: Antibacterial activity of the ethanolic extract of *Nepetaseptemcrenata*:

Microorganism	Ethanolic extract				Standard (ref. 10 ug)
	10 ug	100ug	1mg	100mg	++
Staphylococcus S.aureus	00	00	00	00	++
E. coi	00	00	00	00	

+:very weak inhibition, ++: strong inhibition, 00: inhibition zone were not detected

Table 6: Effect of interperitoneal injection of ethanolic extract of *Nepetaseptemcrenata*with two doses (1/5 LD_{50}& 1/10 LD_{50}) on plasma lipid peroxidation content (µmol MDA/L) of rat.

Time period	Control	Treatment			
		1/5 LD_{50}	% of change	1/10 LD_{50}	% of change
2hours	0.365±0.01[a]	0.314±0.03	-13.97	0.334±0.01	-8.49
4hours		0.270±0.01*	-26.03	0.358±0.01*	-1.91
8hours		0.267±0.02*	-26.85	0.249±0.02*	-31.78
16hours		0.288±0.03	-21.10	0.341±0.01	-6.57
24hours		0.357±0.01*	-2.19	0.264±0.019*	-27.67

[a]Represents means ±SE of 6 rats/group.
*Significant difference as compared with the control group using student's unpaired "t-test" at p<0.05.
*Significant difference between the two treated does at the same time using Student's unpaired "t-test" at p<0.05.
*Significant difference between control and the five treated groups (in each dose 1/5 LD_{50}&1/10ld_{50})using one-way ANOVA, p<0.05.

Table 7: Effect of interperitoneal injection of ethanolic extract of *Nepetaseptemcrenata*with two doses (1/5 LD_{50}& 1/10 LD_{50}) on Blood glutathione content (mg/dl) of rat.

Time Period	Control	Treatment			
		1/5 LD_{50}	%of change	1/10 LD_{50}	% of change
2hours	1152.23±1.85[a]	2150±24.6*	+86.59	1859.25±12.1678	+61.36
4hours		1155.55±7.40	+0.28	1156.70±2.94	+0.38
8hours		1193.66±27,42	+3.59	1312.79+27,48	+13,93
16hours		1404.86±23.79*	+21.92	1375.8±27.48	+19.40
24hours		1170.95±4.74*	+1.62	1504.93±58.92*	+30.61

[a]Represents means ±SE of 6 rats/group.
*Significant differ5ence as compared with the control group using student's unpaired "t-test" at $p<0.05$.
*Significant difference between the two treated does at the same time using Student's unpaired "t-test" at $p<0.05$.
*Significant difference between control and the five treated groups (in each dose 1/5 LD_{50}&1/10ld_{50}) using one-way ANOVA, $p<0.05$.

Table 8: Effect of interperitoneal injection of ethanolic extract of *Nepetaseptemcrenata*with two doses (1/5 LD_{50}& 1/10 LD_{50}) on Blood catalase activity (µ/ml) of rat.

Time period	Control	Treatment			
		1/5 LD_{50}	% of change	1/10 LD_{50}	% of change
2hours	10.8±1.98[a]	19.8±1.98*	+83.33	16.20±1.105	+50
4hours		14.4±1.29	+33.33	21.6±2.7*	+100
8hours		23.4±1.43*	+116.6	26.64+1.41*	+146.66
16hours		37.8±1.58*	+250	25.75±2.88*	+138.33
24hours		21.6±2.60*	+100	28.26±3.0*	+161.66

[a]Represents means ±SE of 6 rats/group.
*Significant differ5ence as compared with the control group using student's unpaired "t-test" at $p<0.05$.
*Significant difference between the two treated does at the same time using Student's unpaired "t-test" at $p<0.05$.
*Significant difference between control and the five treated groups (in each dose 1/5 LD_{50}&1/10ld_{50})using one-way ANOVA, $p<0.05$

Table 9: Effect of interperitoneal injection of ethanolic extract of *Nepetaseptemcrenata*with two doses (1/5 LD_{50}& 1/10 LD_{50}) on (Cu/Zn) superoxide dimustase activity (µ/ml blood)of rat.

Time period	Control	Treatment			
		1/5 LD_{50}	% of change	1/10 LD_{50}	% of change
2hours	22.5± 2.3[a]	32.61±2.5*	+44.93	72.79±2.80*	+223.51
4hours		50.72±4.81*	+125.42	50.89±5.90*	+126.17
8hours		90.17±7.27*	+300.75	139.98+9.87*	+522.13
16hours		109.34±4.71*	+385.95	74.63±3.18*	+231.68
24hours		78.52±4.21*	+248.97	81.22±2.57*	+260.97

[a]Represents means ±SE of 6 rats/group.
*Significant differ5ence as compared with the control group using student's unpaired "t-test" at $p<0.05$.
*Significant difference between the two treated does at the same time using Student's unpaired "t-test" at $p<0.05$.
*Significant difference between control and the five treated groups (in each dose 1/5 LD_{50}&1/10ld_{50})using one-way ANOVA, $p<0.05$.

Table 10: Effect of interperitoneal injection of ethanolic extract of *Nepetaseptemcrenata*with two doses (1/5 LD_{50}& 1/10 LD_{50}) on (Cu/Zn) superoxide dimustase activity (µ/ml blood)of rat.

Time period	Control	Treatment			
		1/5 LD_{50}	% of change	1/10 LD_{50}	%of change
2hours	66.45±.52[a]	66.73±0.5*	+0.42	79.66±1.7*	+19.88
4hours		68.52±3.3*	+3.11	78.43±1.95*	+18.03
8hours		68.085±1.95*	+2.46	91.6±3.05*	+37.84
16hours		84.63±2.29*	+27.35	72.18±3.42*	+8.62
24hours		95.43±1.05*	+45.61	69.16±4.32*	+4.08

[a]Represents means ±SE of 6 rats/group.
*Significant differ5ence as compared with the control group using student's unpaired "t-test" at $p<0.05$.
*Significant difference between the two treated does at the same time using Student's unpaired "t-test" at $p<0.05$.
*Significant difference between control and the five treated groups (in each dose 1/5 LD_{50}&1/10ld_{50})using one-way ANOVA, $p<0.05$.