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Effects of Application of Chlorpyrifos Ethyl and Rose Water on Rat Pancreas

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Abstract:

This study was conducted to detect preventive effects of rose water against toxicological effects of chlorpyrifos-ethyl. Group I was control group, group II was chlorpyrifos-ethyl (CPE) group (0.3 mg/kg/day), group III was water solution of rose extract (WR) group (100 mg/kg/day) while group IV was water solution of rose extract + chlorpyrifos-ethyl group (WR+CPE). The rats were sacrificed after fifteen days of experiment to collect blood samples. The results showed that comparing to the CPE group, there was a significant ($p<0.001$) decrease in the malondialdehyde (MDA) and total oxidant capacity (TOC) levels and a significant ($p<0.001$) increase in the total antioxidant capacity (TAC) and insulin levels in the CPE+GS group. Between the control and WR groups, insulin, WBC, catalase (CAT), lipase and amylase levels were significantly ($p<0.001$) higher, while TOC and OSI levels were significantly ($p<0.001$) lower in the WR group. The highest OSI levels were determined in the CPE group followed by CPE+RW (1.02 µmol H_2O_2 equ. /L), control (0.93 µmol H_2O_2 equ. /L) and RW (0.87 µmol H_2O_2 equ. /L) groups. In conclusion, it can be said that WR showed positive effects on the enzymes of pancreas and hematological parameters in rats, and it reduced in toxic effects of CPE.

Key Words: Chlorpyrifos-ethyl, Malondialdehyde (MDA), Total antioxidant capacity (TAC), Total oxidant capacity (TOC)

Introduction:

Pesticides are substances used for prevention of unwanted insects, rodents, plants, moss and other harmful organisms. Extensive and misuse of them can cause negative effects on human and environment. Pesticides used widely in agriculture cause to occurrence of reactive oxygen radicals such as hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl (-OH). These antioxidants cause to oxidative stress if they cannot be removed by immune system and a result of oxidative stress, DNA damage and pathological cancer lesions can be seen [1,2]. Chlorpyrifos-ethyl (CPE; 0,0´- diethyl 0-[3,5,6-trichloro-2-pyridyl] phosphorothionate) is an organophosphate insecticide widely used against pests of garden, agricultural and forest that people are frequently exposed [3]. Like in other organophosphates, main principle of the effect of this pesticide is phosphorylation of acetylcholinesterase and following inactivation [4]. CPE is absorbed easily in rats by oral route [5,6]. In Turkey, growing Rosa damascena Mill (Rosaceae) dates back to sometimes before Turkish Republic and it is one of the main commercial agricultural products grown and harvested in Isparta District. Several different commercial products such as water solution of rose extract, rose essential oil, rose jam, rose soap, rose skin and body lotion etc. are produced from Rosa damascena Mill. It is known that Rosaceae family has many species used for medicinal purposes, of which show effects on physiological functions, biochemical activities, and have anticancer, anti-inflammatory, and antioxidant effects. It has been detected that water solution of rose extract and rose essential oil produced from Rose damascena contain several terpenes, antosianin, and flavonoids [7-9]. In this study, preventive effects of water solution of rose extract against toxicological effects of CPE have been investigated in pancreas tissues.

Experimental:

Production of Water Solution of Rose Extract:

Rose flowers [Rosa damascena Mill var. trigintipetala (Dieck)] used in the study belongs to the Keller Taxonomy. Rose flowers (8 kg) were picked up at early hours of day in the second week of June in 2013 and brought to the factory. They were distilled in a classic traditional village distillery which is called as 'Imbik' in Güneykent Town of Isparta district. To achieve that 24-liter water was added to the boiler of distillery and joint device was covered by a special mud. Distillation took two and half hours. The resulting water solution of rose extract contains a small amount of rose essential oil and it is therefore called as 'Rose water oil' and was stored in a cool and dark place.

Experimental Animals:

Thirty-Two Wistar albino male rats (250-300 g each) at age of 8-12 months were used in the study. The rats were accommodated in 30×55×35 cm steal cages at 18-25^{0}C. Water was available ad libitum. Prior to experiment, the rats were kept a month to get them adapted to the new laboratory conditions. The rats were randomly divided into four experimental groups each group having eight rats. Group I was Control group and the rats in this group were supplied with tap water and standard rat feed. In Group II (CE group), the rats were supplied with tap water, standard rat feed and 0,3 mg CPE (Dursban 4, Dow AgroSciences) was given [(10)] individually once in a day for 15 days by oral gavage. In Group III, the rats were supplied with tap water, standard rat feed and 1 mL water solution of rose extract was given individually once in a day for 15 days by oral gavage. In Group IV, the rats were supplied with tap water, standard rat feed and 1 mL water solution of rose extract + 0,3 mg CPE (Dursban 4, Dow AgroSciences) mixture was given individually once in a day for 15 days by oral gavage. The rats were withdrawn from feeding for 2 hours after each application. At the end of 15 days, the rats were anesthetized by intra-muscular injection of 10% Ketamine (Alfamin, Alfasan IBV)-2% xylazin and were decapitated in groups of eight to collect pancreas samples.

Biochemical and Hematological Analysis:

The blood samples were centrifuged at 3000 rpm/min for 15 min to obtain blood serum and insulin levels were measured by using an auto-analyzer (Olympus, AU 5200). Lipase, amylase activities and amylase and lipase activities were determined by using an auto-analyzer (Abbott Aeroset, IL, USA). Hemogramme was performed by using a hematological analyzer (BeckmanCounter LH 780).

TAC and TOC Measurements:

Total antioxidant capacity (TAC) levels were measured by Erel's TAC method [(11)] which is based on the bleaching of the characteristic colour of a more stable 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radical caution by antioxidants. The results were expressed in mmol Trolox equiv./L. Total oxidant status (TOS) serum concentrations were measured using Erel's TOS method [(12)], which is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidative species in acidic medium and the measurement of the ferric ion by xylenol orange. The results were expressed in μmol H_2O_2/L. Erel's TAC and TOS methods are colorimetric and automated and the precision of this assay is excellent – lower than 3% [13,14]. The percentage ratio of total peroxide level to TAC level was taken as the oxidative stress index (OSI). To perform the calculation, the unit of TAC, mmol Trolox equivalent/L, and the OSI value was calculated using the following formula; OSI = [(Total peroxide, mmol/L)/ (TAC, mmol Trolox equivalent/L)/100] [(14-16)].

CAT Measurement:

CAT activity was detected according to Aebi method 18. CAT catalysis deterioration of H_2O_2 to water and molecular oxygen. In the study; CAT activity was detected spectrophotometrically at 240 nm according to the decrease in H_2O_2 concentration during a unique time. CAT activities were detected by UV spectrophotometric method, which depends on the breaking into pieces theory by CAT. CAT activity expressed as kU/g protein.

MDA Measurement:

To measure MDA, one of the lipid peroxidation products, Draper and Hadley double heating method was used [19]. The principle of the method depends on the measurements of the absorbance of MDA-TBA complex at 532 nm. Results were given as nmol/g.

Gas Chromatography (GC) and Gas Chromatography–mass Spectrometry (GC/MS):

The concentrated diethyl ether extracts of rose water and essential oil samples were subjected to GC analyses on a Nucon gas chromatograph model 5765 and Perkin Elmer Auto XL GC equipped with flame ionisation detector (FID) and two stationary phases of different polarity, viz. BP-20 (30m×0.25mm×0.25 mm film thickness) and DB-5 (30m×0.32mm×0.25 mm film coating) fused silica columns, respectively. Hydrogen was the carrier gas

at 1.0 mLmin-1. Temperature programming was done from 70°C to 230°C at 4°C min-1 with an initial and a final hold time of 2 min (for BP-20) and from 60°C to 210°C at 3°C min-1 (for DB-5). The injector and detector temperatures were 210°C and 230°C on BP-20 and 210°C and 220°C on DB-5 column, respectively. The injection volume was 0.02 mL and the neat and the split ratio was 1: 30. GC–MS was recorded on a Perkin Elmer Auto System XL GC and Turbo Mass Spectrometer fitted with fused silica capillary column, PE-5 (50m×0.32mm id×film thickness 0.25 mm). The column temperature was programmed at 100–280°C at 3°C min1, using helium as a carrier gas at a constant pressure of 10 psi. The injector temperature was 220°C and MS conditions were: EI mode 70 eV, ion source temperature 250°C.

Identification of Compounds:

The compounds were identified by comparing the retention time, retention indices and mass fragmentation pattern with those of literature values, standard compounds that run under the same conditions and by peak enrichment on co-injection with authentic samples wherever possible to corroborate identities [20-25]. The peak area percentage was computed from the peak areas without applying FID response factor correction.

Statistical Analysis:

Non-parametric Mann-Whitney U test was used to evaluate the biochemical and hematological results between the rat groups 26.

Results and Discussion:

Volatile compounds of water rose oil were shown in Table 1. Phenyl ethyl alcohol was, an important konkret compound, found at the level of 22.16% in our sample. Tricosane, tricontante, heneicosane and nonadecane were determined at the levels of %10.89, %12.95, 9.78% and 14.89%, respectively (Table 1). The Chromatogram of standard mixture is shown in Figure 1. A chromatogram of chlorpyriphos ethyl obtained from the blood sample of a rat in group II is shown in Fig. 2. A chromatogram of CPE obtained from the blood sample of a rat in group IV is shown in Figure 3. Calibration function values of studied pesticide (CPE) are given in the Table 2. The mean values of biochemical and hematological results are summarized in Table 3. The mean values of antioxidant and oxidant parameter results are summarized in Table 4. In comparison of ALT enzymes, no significant difference was found between the control and RW groups but a significant ($p<0.001$) reduction was determined between the groups of CPE and CPE + RW. Likewise, the same results were observed for AST and GGT enzymes. This make us to think that rose water oil given with CPE may lead to a preventive reduction of pancreas enzymes. In the comparison of lipase levels between the groups, a significant ($p<0.001$) reduction in the levels of lipase and amylase was determined in the control and CPE + RW groups compared to the CPE group. These results indicate that CPE can be effective on pancreas in rats and rose water oil solution might have reduced the toxicological effect of CPE in the CPE + RW group.

Conclusions:

The R. damascena extract may be exploited as a natural antioxidant and health promoting agent that can conveniently find its appropriate therapeutic applications [25]. Previous studies have shown that R. damascena has a high antioxidant, hepatoprotective and antibacterial effects [27, 28]. In order to evaluate the cellular oxidative stress, total oxidant status (TOS) and total antioxidant status (TAS) are well-established markers in experimental analysis. TOS is defined as composed of radicals, which are taken directly into the body or may be released during some reactions. Total Antioxidant Status (TAS) also means a defense mechanism of the body against oxidant stress. TAS is composed of various enzymes and vitamins 32. In this study, among the four groups the highest OSI levels were determined in the CPE group (1.12 µmol H_2O_2 equ. /L) followed by CPE + RW group (1.02 µmol H_2O_2 equ. /L), control group (0.93 µmol H_2O_2 equ. /L) and RW group (0.87 µmol H_2O_2 equ. /L). In terms of increased TAS, these results indicate that application of rose water oil solution reduces toxicological effects of CPE. In the RW supplemented group, increased TAS and decreased TOS and OSI levels (values) support this. In a study performed on people who were exposed to pesticides for long period, similar results were found in terms of TAS, TOS and OSI [30,31]. In this study, the WBC levels were found significantly ($p<0.001$) lower in the CPE and CPE + RW groups compared with the control group. Similarly, Ambali [(32)] and coworkers (2010), found a significant reduction in the levels of WBC in the blood samples of rats given CPE. It is eye catching that the WBC level is significantly higher in the CPE + RW group compared to the CPE group. Furthermore, the level of WBC in the RW group is higher than control group. When the PLT levels were compared between the groups, similar results were observed as in the WBC levels. Published literature exists showing CPE application causes to the significant changes in the PLT levels in rats [29, 32]. As a result of this study,

it can be concluded that rose water oil solution application shows a positive preventive effect against the toxicological harm of an organophosphate pesticide, CPE. Particularly, significant reductions in the levels of pancreas enzymes (lipase and amylase) and significant increase in the WBC, TAK levels indicate us to reach to this conclusion. Considering rose water being accepted as a food ingredient in recent years, consumption of rose water in terms of its antioxidant content may show positive effects on health.

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Table 1: Volatile compounds of water rose oil (%).

Compounds	**%**
Caryophyllene	0.00
Diacetone alcohol	0.00
Heptadecane	1.33
Germacrene-D	0.40
Geranyl acetate	0.31
Citronellol	1.81
Nerol	2.33
Acetic acid, Phenylethyl ester	0.65
Geraniol	3.37
Benzyl alcohol	0.57
Nonadecane	14.89
9-Nonadecane	3.09a
Phenyl Ethyl Alchool	22.16
Eicosane	1.18
Heneicosane	9.78
Tricosane	10.89
Tricontante	12.95
Tetradecanol	0.73
1-octanyl-4-ol	1.23a
2-ethyl-2-methyl-1,3-propandiol	1.07
1-octanol,2,2, dimethyl	1.20
Concrete yield (%)	0.34

Table 2: Calibration function values of CPE.

Bileşikler	r^{2*}	LOD** (ng/l)	LOQ*** (ng/l)	Recovey (%)	Linear range (ng/l)	RSD**** (%)
CPE	0.999	20.0	66.0	100	20.0–1000	1.00

* Correlation value
** Detection limit
*** Lower limit of determination
**** Relative standard deviation

Table 3: Mean values of biochemical and hematological blood parameters of experimental groups.

	Lipase* (U/L)	**Amylase*** (U/L)	**WBC*** ($\times 10^6$ mm^3)
Control	13.6 ± 5.4	1856.5 ± 224.3	9.75 ± 2.08
CPE	21.6 ± 6.7	2144.8 ± 242.2	5.46 ± 1.58
CPE + RW	17.5 ± 5.9	1930.9 ± 231.6	7.48 ± 1.95
RW	14.1 ± 5.6	1789.4 ± 218.1	12.72 ± 3.29

*****Significant

Table 4: Mean values of antioxidant and oxidant parameter results of experimental groups.

	TAS* (µmol H_2O_2 equ. /L)	**TOS*** (µmol H_2O_2 equ. /L)	**OSI*** (TOS/TAS)	**MDA*** (nmol/g)	**CAT*** (kU/g)
Control	1.58 ± 0.18	1.47 ± 0.15	0.93	2.68 ± 0.85	21.63 ± **6**.24
CPE	1.75 ± 0.21	1.97 ± 0.33	1.12	5.74 ± 1.79	13.55 ± **4**.89
CPE + RW	1.63 ± 0.20	1.67 ± 0.19	1.02	3.40 ± 1.05	17.41 ± **5**.12
RW	1.71 ± 0.23	1.50 ± 0.17	0.87	2.54 ± 0.79	28.74 ± **8**.06

*****Significant

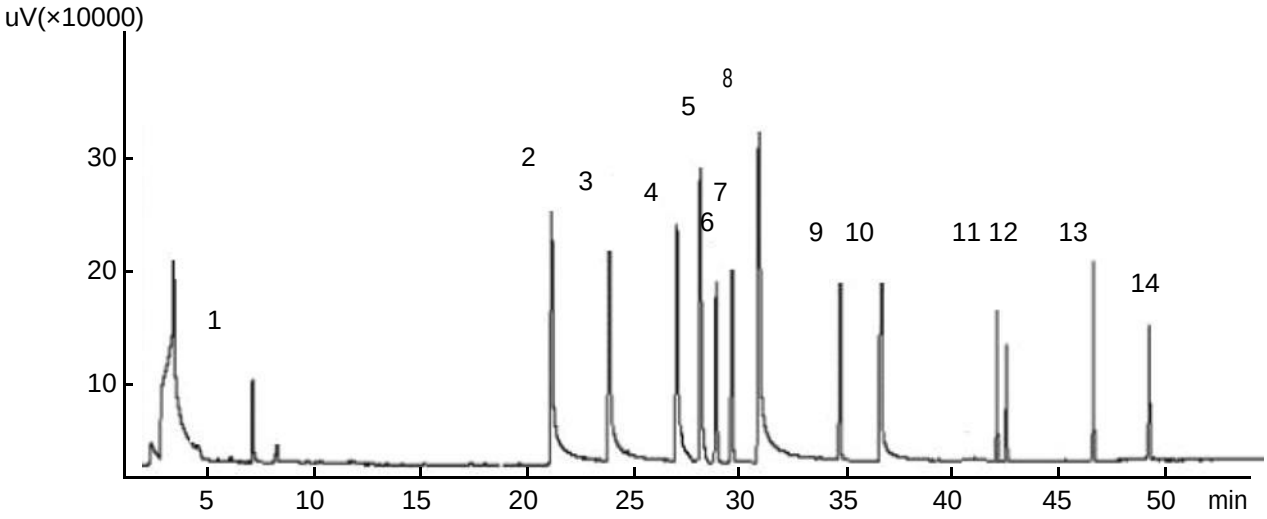

Figure 1. Chromatogram of standard mixture (3–5 µg/L) and pesticides (GC/NPD). 1—dichlorvos; 2—diazinon, 3—parathion_ methyl, 4—chlorpyriphos ethyl, 5—malathion, 6—cyprodinil, 7—captan, 8—methidathion, 9—kresoxim_methyl, 10—ethion, 11—azinphos ethyl, 12—fenazaquin, 13—cypermethrin, 14—deltamethrin (TRB–5, 30 m × 0.32 mm, 0.25 µm).

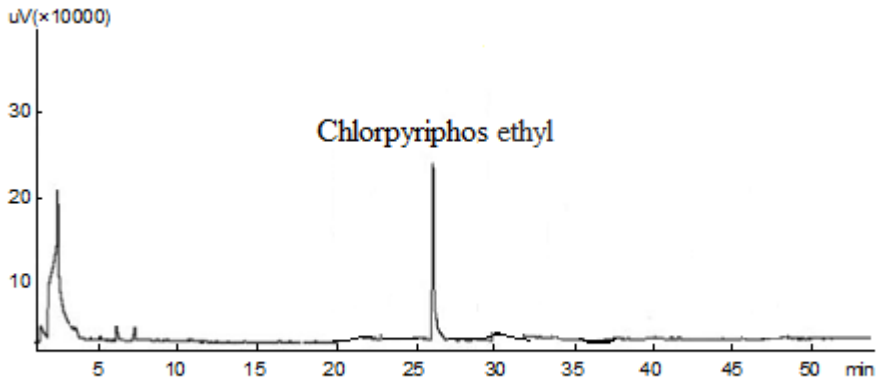

Figure 2. Chromatogram of chlorpyriphos ethyl in a blood sample of rat in group II.

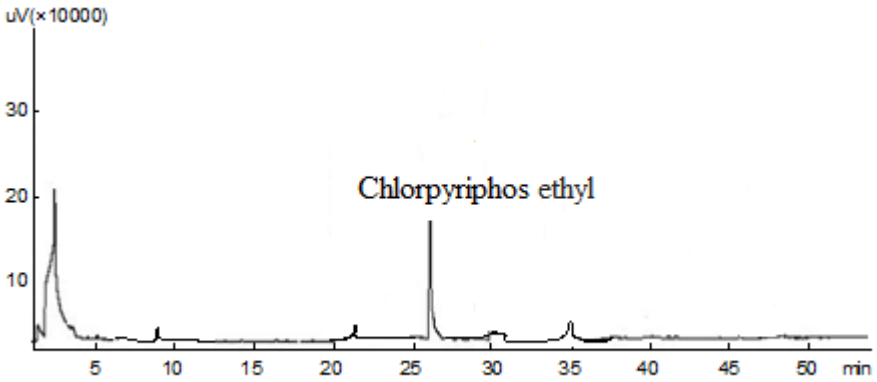

Figure 3. Chromatogram of CPE in the blood sample of a rat in group IV.