The Protective Effect of Rosewater against DNA Damages and Oxidant and Antioxidant Parameters of Rats Exposed to Chlorpyrifos Ethyl

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Objective: The aim of this study was to determine both the protective effect of rose water (RW) against DNA damage in the tissues of rats exposed to chlorpyrifos-ethyl (CPE) and RW's effect on the oxidant and antioxidant levels in the blood serum and brain tissues of those same rats.

Methods: In this experimental study, 32 mature male wistar albino rats were divided into 4 groups: group I, control; group II, CPE; group III, RW; and group IV, CPE+RW. The parameters of DNA tail intensity and DNA tail moment were analysed in blood samples by comet assay. Glutathione-S-transferase (GST), catalase (CAT), and malondialdehyde (MDA) levels in brain tissues were examined. In blood serum, the levels of melatonin (MT) from 3-nitrotyrosine (3-NT) were determined.

Results: In the CPE+RW group, the MDA and 3-NT levels in the brain tissues were significantly reduced (p<0.001), while the MT, GST, and CAT levels were significantly higher (p<0.001) compared to those of the CPE group. When the control and RW groups were compared, the CAT, GST, and MT levels were significantly higher (p<0.001) in the RW group, while the MDA and 3-NT levels were significantly lower (p<0.001).

Conclusion: In rats, RW had positive effects on oxidant damage created by CPE. Both the DNA tail intensity and DNA tail moment in the CPE group were significantly higher (P<0.001) compared to those measures for the control group. [*P R Health Sci J 2019;38:113-117*]

Key words: Antioxidant, Oxidant, Rosewater, Chlorpyrifos ethyl, Tail moment

Human health is affected by environmental, environmental-biological (microorganisms), and physical factors, of which last nutritional deficiency forms a significant part. Classified as a chemical factor, pesticides influence human health and can lead to significant chronic or acute complications (1,2). Widely used in agriculture, pesticides can lead to the formation of reactive oxygen types, such as hydrogen peroxide (H_2O_2), superoxide (O_2-•), and hydroxyl radical (•OH). These radicals may react with biological macromolecules; may induce enzyme inactivation and cause DNA damage. When accumulated in adipose tissue, pesticides may result in the peroxidation of polyunsaturated fatty acids (PUFA) (3).

If not taken away by the antioxidant defence system, these oxidants will cause oxidative stress. Oxidative stress can cause DNA damage, itself leading to any number of pathological conditions, including cancer (3,4). The organophosphate insecticide CPE (O,O'-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate) is widely used in agriculture. The role of oxidative stress in toxicity induced by CPE has been proven by studies (5-7).The aim of this study was to determine both the protective effect of rose water (RW) against DNA damage (as evidenced by tail intensity and tail moment) in blood sampled from rats exposed to chlorpyrifos-ethyl (CPE) and RW's effect on the oxidant and antioxidant levels in those same samples.

Methods

The roses (Rosa damascene Mill var. trigintipetala [Dieck]) used in the study belonged to the Keller taxonomy. Eight kg of rose blooms were extracted from the rose plants that were picked in the early morning to sell to factories. They were distilled in a traditional village distillery, using what is called an "imbik (distillation boiler) in the municipality of Güneykent in the province of Isparta, Tukey. To do so, 24 L of water was added

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The author/s has/have no conflict/s of interest to disclose. Funding: This work was supported by the Adnan Menderes University ADU/BAP-13001 Project.

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to the boiler of the distillery and a joint device was covered by a special mud. The distillation took 2.5 h. The resulting water solution of rose extract contained a small amount of rose essential oil (i.e., rose water oil), which was stored in a cool and dark place (7).

The animal materials used in this study were male wistar albino rats (32), which weighed 250 to 300 grams each, and which were 8 to 12 months old. The animals were kept in steel rat cages, measuring 30×55×35 cm; the temperature of the room rangedfrom18 to 25°C. The rats were given ad libitum access to tap water and rat food. The experiment started 1 month after the rats were brought to the laboratory, to ensure the adaptation of the rats to the new environment. The rats were randomly divided into 4 groups: group I (the control group), group II (the chlorpyriphos-ethyl [CPE] group), group III (the RW group), and group IV (CPE+RW group). The rats in the different experimental groups that received added substances received them at the same times every day, always 2 hours before being fed, thereby prevent their food from interacting with the added materials and affecting the results. Experimental animals were housed individually and maintained on a 12-hour light–dark cycle (lights on from 8:00 to 20:00 h). All the experimental procedures were performed during the light part of the cycle, at the same time each morning, in the home animal cages according to groups.

The study period of the research was 15 days. During the study, the animals in group I received tap water and standard rat food; those in group II received tap water and standard rat food plus 0.3 mg/kg/day of CPE (Dursban 4, Dow AgroSciences); group III rats received tap water and standard rat food plus 100 mg/kg/day of RW; group IV rats received tap water and standard rat food plus 100 mg/kg/day of RW and 0.3 mg/kg/day of CPE. At the end of 15 days, the rats were decapitated under anaesthesia with 10%ketamine hydrochloride (HCl) (Alfamine-IM), and 2%xylazineHCl (Rompun-IM), and that part of the study was concluded.

The brain tissues of the animals were isolated within 3 minutes. The obtained brain tissues were washed with cold (+4°C) sodium chloride 0.9% (NaCl) and were dried with blotting paper. All the brain tissues were kept at -20°C until the day of the experiment; on the day of the experiment they were homogenized with 0.05 M sodium-phosphate buffer, which has a1:10 weight/volume ratio (w/v)contains 0.25 M sucrose, and has a 7.4 pH; this process took place in a homogenizer (10000 rpm for 3 minutes).

The blood samples taken to the laboratory were centrifuged with a cold centrifuge at +4°C (1000g) for 15 minutes. The obtained plasma was put into Eppendorf tubes and kept at -80°C. MT levels were measured using an MT radioimmunoassay (RIA) Kit for rats.

The competitive 3-NT ELISA Kit was used to determine protein peroxidation in plasma. The absorbance was measured as 450 nm on a microplate reader. The results were defined as μmol/mg of protein in proportion to their protein contents.

The measurement of CAT activity was done according to the method described by Aebi (8). It depends on an observation of the enzymatic breakdown of the H2O2 substrate with CAT at 240 nm. In the study, CAT activity was defined as unit/mg (U/mg) of protein.

The principle of the experiment was based on the measurement of the pink-red color of the compound formed by MDA as an end product of the peroxidation of polyunsaturated fatty acids with thiobarbituric acid (TBA) in a hot environment (9).

GST enzyme activity was detected by the measurement of the thioether bond formation between the glutathione substrate and GST by spectrophotometer at 340 nm (10).

The comet assay used here was adapted from a method described previously (11). For our study, fresh blood samples were mixed with a PBS solution to determine the DNA fragmentation of the blood lymphocytes. Lymphocytes were isolated with histopaque and suspended in a freezing medium. Isolated lymphocytes were slowly frozen in aliquots of 1 ml at -80°C. Conventional end-frosted slides were pre-coated with 1% normal melting agarose. This suspension was mixed with pre-warmed low-melting-point agarose. The positive control slides were dipped in an H2O2 solution for 5 min at 4°C. Following lysis, the slides were aligned in a horizontal gel electrophoresis tank (CSL-COM20, Cleaver Scientific, UK) that was connected to a recirculating cooler (FL300, JULABO, Germany) set at 4°C and filled with freshly made alkaline electrophoresis solution. Electrophoresis (CS-300V, Cleaver Scientific, UK) was carried out at approximately 1 V/cm for 20 min. The measurements of the tail intensity and tail moment of comets were made using a computer-based image analysis system (Comet Assay IV, Perceptive Instruments, UK). The mean value of the percent of tail DNA and the mean tail moment parameters were calculated and used to assess the DNA damage.

This study was approved by the Adnan Menderes University experimental animals ethics committee (protocol number B.30 .2.ADÜ.0.00.00.00/050.04/2012/094).

For the statistical analysis, SPSS version 17 (SPSS Inc. Released 2008.SPSS Statistics for Windows, Version 17.0.Chicago: SPSS Inc.) was used. A nonparametric Mann-Whitney U test was used for the evaluation of the biochemical and hematologic parameters of the different groups of rats. Statistical significance was assumed with a p value less than 0.05.

Results

The essential oil components of RW are presented as percentages in Table 1. Phenyl ethyl alcohol, which is an important component, was found to be 22.16% in our study. The tricosane, triacontane, heneicosane, and nonadecane levels were measured, respectively, as 10.89%, 12.95%, 9.78%, and 14.89%. Methyl eugenol, an undesired component for the purposes of our study, was not detected in our samples. These study results are consistent with those in the literature.

Table 1. The essential-oil components of RW (%).

Components	
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 alcohol	22.16
Eicosane	1.18
Heneicosane	9.78
Tricosane	10.89
Triacontane	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 efficiency (%)	0.34

The average levels of the antioxidants and oxidants detected in the blood samples of the rats are presented in Table 2. In the CPE+RW group, the MDA and 3-NT levels in the brain tissues were significantly reduced ($p<0.001$), while the MT, GST, and CAT levels were significantly higher($p<0.001$) compared to those of the CPE group. When the control and RW groups were compared, the CAT, GST, and MT levels were significantly higher ($p<0.001$) in the RW group, while the MDA and 3-NT levels were significantly lower ($p<0.001$).

The effects of CPE and RW on the tail moment and tail intensity of DNA are presented in Table 3. Both the DNA tail intensity and DNA tail moment in the CPE group were significantly higher ($P<0.001$) than they were in control group. When the CPE+RW and CPE groups were compared, both the DNA tail intensity and DNA tail moment were found to be significantly ($P<0.001$) lower in the CPE+RW group.

A chromatogram of standard compounds is given in Figure 1.

In Figure 2, there is a chromatogram of a sample taken from the CPE group of rats.

Calibration function values of CPE were found as follows; r2 (correlation value): 0.999, LOD [limit of detection (ng/l)]: 20.0, LOQ [Lower limit of determination (ng/l)], recovery: 100 %, linear range (ng/l): 20.0-1000, RSD [(relative standard deviation (%)]: 1.00.

Table 2. The average levels of antioxidants and oxidants in blood samples of rats.

	MDA (nmol/mg protein)	CAT (U/mg protein)	GST (U/mg protein)	MT (pg/mL)	3-NT (nmol/L)
Control	0.64 ± 0.05	687.44 ± 77.65	1.14 ± 0.10	2.25± 0.82	2.24± 0.41
CPE	2.14 ± 0.15	357.25 ± 58.35	0.67 ± 0.07	1.26± 0.58	3.39± 0.60
CPE+RW	1.63 ± 0.12	501.47± 41.72	0.88 ± 0.09	1.68± 0.63	2.83± 0.38
RW	0.54 ± 0.04	774.15 ± 87.49	1.25 ± 0.10	2.44± 0.88	2.02± 0.39

Table 3. Effects of CPE and RW on tail moment and tail intensity of DNA.

Group	n	Parameters	
		Tail Moment	Tail Intensity (%)
Control	8	7.18 ± 0.95c	25.87 ± 2.12c
RW	8	10.84 ± 1.87b,c	30.96 ± 2.50b,c
CPE	8	26.94 ± 1.60a	55.50 ± 3.09a
CPE+RW	8	14.88 ± 1.95b	36.13 ± 3.24b
P		P<0.001	P<0.001

a, b, c Different letters in the same column indicate significant change statistically.

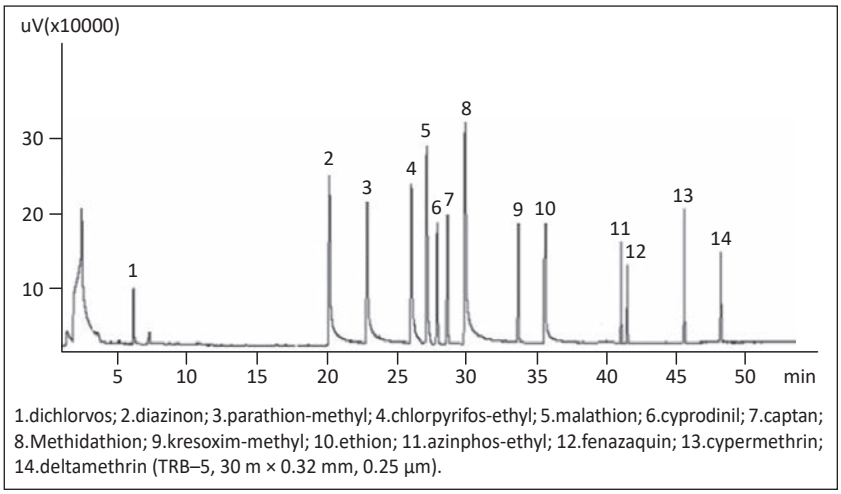
Discussion

It is known that extracts obtained from rose pulp made from fresh roses have high antioxidant and antibacterial effects (12,13). The results of this study also show the antioxidant property of RW. Higher levels of antioxidants (CAT, GST, MT) were seen in the rats that received RW than were seen in those that did not (Table 2).

MT is a very strong antioxidant hormone. It is released from the pineal gland in the dark (at night, under normal circumstances) and has a regulatory role in many biological functions, including sleep, reproduction, the circadian rhythms, and immunity. Another important biological function of MT is as a free-radical scavenger (14). Some toxic elements, such as pesticides, may change the antioxidant properties of MT (15,16). In this study, it was observed that the MT levels in the rats that received CPE were significantly decreased in comparison with those levels in the rats of the control and RW groups. Our conclusion was that the pesticide CPE reduces MT levels, while RW increases them.

In this research, the rats in all the groups lived under the same light-dark cycle (lights on from 8:00 to 20:00 h). When comparing the MT levels, a significant difference was found between those levels in the rats in the CPE group and those in the RW group. This result suggests that CPE administration negatively affects MT levels in rats. To the contrary, the application of RW caused an increase in MT levels.

Ensuring the interaction between glutathione and electrophilic and hydrophilic compounds, GSTs are members of the phase-II detoxification enzyme family that protects cellular macromolecules against reactive electrophiles (17). In the present study, it was shown that after 15 days of CPE administration, a significant decrease in GST levels was observed in the CPE group of rats compared to those in the control group. Another important result was that the GST levels of the RW group were significantly higher than were those of the control group. RW administration led to increases in the levels of GST. Again, when compared to such levels in the animals in the CPE group, the GST levels of those in the CPE+RW group were significantly higher.



1.dichlorvos; 2.diazinon; 3.parathion-methyl; 4.chlorpyrifos-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 1. Chromatogram of standard solutions GC/NPD of pesticides (3–5 µg/L).

Several studies have shown that pesticides reduce CAT activity (18–20). Similarly, in this study, we found that the pesticide CPE reduces CAT activity in rats. However, it was found that the effects of CPE on the animals in the CPE+RW group were reduced significantly by RW. CAT activity was found to be relatively high in the rats in the RW group compared to such activity in the control-group animals. The administration of RW to the rats reduced the levels of the oxidant parameters MDA and 3-NT.

Several studies performed have shown that organophosphate pesticides, such as CPE, increase the levels of MDA, the product of lipid peroxidation (2,21). A significant increase in the MDA levels of the rats in the CPE group was also observed in this study. RW administered together with CPE caused significant decreases in the MDA levels of the animals in the CPE+RW group compared to those in the CPE group.

Nitrotyrosine is formed by the interaction of peroxynitrite with the tyrosine residues of proteins, and it can be used to evaluate the potential cytotoxic effects of nitric oxide. The formation of nitrotyrosine represents a peroxynitrite-mediated protein modification and differs from other reactive oxygen type-mediated modifications (22). In our study, it was shown that CPE interacted with proteins in rats and causes damage. Likewise, in our analysis, it was shown that RW administration reduced this damaging effect.

On comparing the results of the statistical analysis (together with the increased DNA tail intensity and DNA tail moment) of the control group with those of the CPE group, we determined that that CPE had an adverse effect. Similar results were observed in a study conducted by Mehta et al. (23) when the animals in a CPE-RW group were compared with those in a CPE group; both DNA tail intensity and DNA tail moment were observed to be significantly lower in the rats in the CPE-RW group. These results indicate that RW decreases the DNA damage caused by CPE.

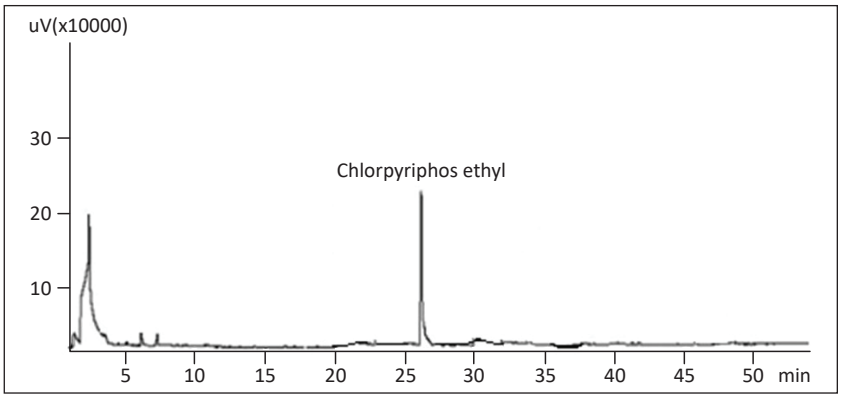


Figure 2. Chlorpyrifos-ethyl chromatogram of a blood sample taken from a rat from group II.

Conclusion

As a result, in this study, in which we evaluated the antioxidant properties of RW in terms of its being used against the oxidative stress caused by pesticides, we were able to show that RW has a protective effect. With this study, it is proven that RW, which is consumed as a nutrient in some countries, is also a good source of antioxidants. As shown in this study, RW administration caused an increase in antioxidant parameters and reduced the oxidant parameters. In addition to this, administering RW to the rats in our study led to decreases in DNA damage (as measured by tail intensity and tail moment).

Resumen

Objetivos: El objetivo de este estudio fue determinar el efecto protector del agua de rosas (AR) contra los daños del ADN y los parámetros oxidantes y antioxidantes en el suero sanguíneo y los tejidos cerebrales de ratas expuestas al clorpirifós etil (CE). Métodos: En este estudio experimental se dividieron 32 ratas wistar albino macho maduras en cuatro grupos como:grupo I, control;grupo II,CE;grupo III,AR y grupo IV,CE+AR. Los parámetros de la intensidad de la cola del ADN y el momento de la cola del ADN se analizaron en muestras de sangre mediante ensayo cometario. En los tejidos cerebrales se examinaron glutatión-S-transferasa (GST), catalasa (CAT) y malondialdehído (MDA). En el suero sanguíneo se examinaron melatonina (MT) a partir de 3-nitrotirosina (3-NT). Resultados: En los grupos CE y AR, los niveles de MDA y 3-NT en los tejidos cerebrales se reducen significativamente ($p<0.001$), mientras que los niveles de MT, GST y CAT son significativamente

altos (p<0.001); cuando se comparan con el grupo CE. Cuando se comparan los grupos de control y AR, los niveles de CAT, GST y MT son significativamente altos (p<0.001) en el grupo AR, mientras que los niveles de MDA, 3-NT se reducen significativamente (p<0.001). Conclusión: AR tiene efectos positivos sobre el daño oxidante creado por CEen ratas. Tanto la intensidad de la cola del ADN como el momento de la cola del ADN en el grupo CEson significativamente altos (P<0.001), en comparación con el grupo control.

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