Original Article

Histopathological Study of Protective Effects of Honey on Subacute Toxicity of Acrylamide-Induced Tissue Lesions in Rats' Brain and Liver

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ABSTRACT

Background: The therapeutic potential of honey is related to antioxidant activity against reactive oxygen species because it contains compounds such as polyphenols; therefore, we evaluated the potential protective effect of honey on subacute toxicity of ACR by histopathologic study on tissue lesions in rat.

Methods: In Ferdowsi University of Mashhad, Mashhad, Iran, 2016, male Wistar rats were divided into 7 groups. To induce toxicity, ACR was injected (50 mg/kg for 11 d) to rats in 5 groups. In treatment groups, rats received three doses of honey 1.25, 2.5, and 5 g/kg in addition to the ACR. The two remaining groups received vitamin E (200 IU/kg) and normal saline as positive and negative control respectively. On the last day, after necropsy, tissue specimens from brain and liver were collected for histopathological studies.

Results: Receiving of ACR caused tissue injuries including degeneration, necrosis, hyperemia, hemorrhage and inflammation in liver; ischemic cell change, hyperemia, hemorrhage and edema in brain tissue. Administration of honey considerably reduced tissue damages caused by ACR, particularly with dosage 5 g/kg.

Conclusion: The severity of tissue lesions caused by the ACR can be reduced by honey, likely through its antioxidant activity. Increasing concentrations of honey will enhance its effectiveness.

Keywords: Acrylamide, Histopathology, Honey, Wistar Rat.

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INTRODUCTION

Acrylamide (ACR) is produced commercially by hydrolyzing acrylonitrile, widely used in industry, for example, to synthesize polyacrylamide [1-3]. Two forms of ACR, monomer, and polymer, exist. The monomer form is a toxic complex and made during unsuitable thermal processing such as frying, baking, and roasting of carbohydrate rich foods [4, 5]. ACR has many harmful effects including neurotoxicity [6,7], genotoxicity[8], carcinogenicity [9,10], reproductive and developmental toxicities [9] and cancer risk [10].

Oxidative stress due to generation of reactive oxygen species (ROS) causes cellular damage via injury of polyunsaturated fatty acids and lipid peroxidation of cell and organelles' membrane. Oxidative stress and dysfunction of mitochondria are the main mechanisms in many chemical-induced cell injuries and neurodegenerative diseases [11].

Histopathologically, acrylamide has injurious effects in various tissues such as brain, liver, kidney, testis, intestine and other organs [5,12,13]. Currently, many studies are prepared to find defensive compounds against the harmful effects of ACR [4, 10, 12, 14, 15].

Honey is a beneficial natural substance and reported as a dietary antioxidant, made from nectar and other sugary substances derived from many plants by honeybees and with therapeutic, traditional, nutritional and industrial value [16, 17].

It contains compounds such as sugars (fructose, glucose), amino acids (e.g. proline), minerals (e.g. potassium), vitamins (vitamin B, C), proteins, organic acids (e.g. gluconic acid), phenolic acids, enzymes (e.g. glucose oxidase), flavonoids,

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carotenoid derivatives and other phytochemicals [18, 19]. Some biological and medicinal properties of honey such as being antioxidant, antimicrobial, anti-inflammatory, anti-fungal, hepatoprotective, hypoglycemic gastro-protective, antihypertensive, immunomodulatory, cardioprotective, neuropharmacological and antitumor effects are well known [17, 19, 20].

Accordingly, honey may improve oxidative stress in various organs and honey, when administered alone or in combination with contractual therapy, may be able to prevent chronic diseases (cancer and cardiovascular diseases) usually associated with oxidative stress [20].

Concerning remarkable role of oxidative stress in ACR-induced toxicity and the antioxidant activity of honey, this research histopathologically investigated the conceivable protective effects of honey on subacute toxicity due to ACR in male Wistar rats' liver and brain.

MATERIALS AND METHODS

This study was done in Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran in 2016. Sterile ACR purchased from Merck (Germany), dissolved in a saline (NaCl 0.9%) solution. Honey was bought from the Osveh Company (Tehran, Iran). By choice, thyme monofloral honey was used. It was first tested to control the purity percentage and its original producer materials in the food quality control laboratory and then different concentrations of honey were checked in vivo rat models. Vitamin E was obtained from the Osveh Company (Iran) in injectable form.

Animals & Housing

Adult male Wistar rats (230 to 250 gr) obtained from the Animal House Faculty of Pharmacy (Ferdowsi University of Mashhad), were kept in colony rooms under conventional conditions with 21± 2 °C temperature, 12/12 h light/dark cycles, and free access to water and standard food, during the experimental period.

All of the animal experiments were carried out in accordance with the Ethical Committee Acts of the Mashhad University of Medical Sciences Mashhad, Iran.

Experimental Protocol

After one week compatibility of rats, the animals were randomly divided into seven groups containing eight rats in each group including; Group 1 (control): normal saline for 14 d, Group 2: 50 mg/kg/d ACR intraperitoneally during 11 d to induce toxicity [21], Group 3: honey (1.25 g/kg, oral by gavage) for 3 d alone and then honey (1.25 g/kg, oral by gavage) + ACR (50 mg/kg, I.P.) for 11 d, Group 4: honey (2.5 g/kg, oral by gavage) for 3 d alone and afterward honey (2.5 g/kg, oral by gavage) + ACR (50 mg/kg, I.P.) for 11 d, Group 5: honey (5 g/kg, oral by gavage) for 3 d alone and then honey (5 g/kg, oral by gavage) + ACR (50 mg/kg, I.P.) for 11 d, Group 6 (positive control): Vitamin E (200 mg/kg, I.P.) at first and then vitamin E (200 mg/kg, I.P.) + ACR (50 mg/kg, I.P.) for 11 d, Group 7: honey (5 g/kg, oral by gavage) for 14 d.

At the end, all rats were euthanized. Tissue samples of brain (cerebrum, cerebellum) and liver were removed immediately after necropsy, excised and fixed in neutral 10% buffered formalin solution.

For histopathological evaluation, the organs were routinely processed by an autotechnicon tissue processor (dehydration, clearing in xylol, embedding with paraffin wax) to prepare 4 to 5-micron thick paraffin sections. Then obtained tissue sections were mounted on glass slides and stained with hematoxylin and eosin stain. Microscopic slides were observed under light microscope. The histopathological damages were scored from zero to three according to table

Table 1. Scorring pattern of histopathological lesions.

Grading of lesion	Description
-	no lesion
+ (Mild)	Lesions in 25% studied microscopic domains
++ (Moderate)	Lesions in 25% to 75% studied microscopic domains
+++ (Severe)	Lesions in more than 75% studied microscopic domains

Statistical Analysis

The values were non-parametric so, the data were analyzed by a Kruskal–Wallis test in order to determine the effects of all groups on each of the experimental parameters. The Mann–Whitney U-test was used to compare the means of each parameter between the groups. Differences were considered statistically significant at $P<0.01$. All Statistical procedures were performed using the SPSS version 21.0 (Chicago, IL, USA).

RESULTS

Histopathological Assessment of Brain Tissues (Cerebrum, Cerebellum) in Rats and Protective Effect of Honey

Results of histopathological assessment and statistical analyses of brain tissues (cerebrum, cerebellum) are shown in Tables 2 and 3.

Table 2. Description and comparison of cerebrum pathological parameters between the groups.

Pathological index	Group	N	Median	Q1	Q3	Min	Max
Hyperemia	ACR	8	2.00	2.00	2.75	1.00	3.00
	H1.25+ACR	8	2.00	1.75	2.00	1.00	2.00
	H2.5+ ACR	8	2.00	1.25	2.00	1.00	3.00
	H5+ ACR	8	2.00	1.25	2.00	1.00	3.00
	Vit E+ ACR	8	1.00	1.00	2.00	1.00	2.00
	H5	8	2.00	1.00	2.00	1.00	2.00
	Normal saline	8	1.00	0.50	1.50	.00	2.00
Hemorrhage	ACR	8	3.00	2.00	3.00	2.00	3.00
	H1.25+ACR	8				3.00	3.00
	H2.5+ ACR	8	2.50	2.00	3.00	1.00	3.00
	H5+ ACR	8	2.00	2.00	3.00	1.00	3.00
	Vit E+ ACR	8	2.00	1.00	2.25	1.00	3.00
	H5	8	2.00	1.00	2.50	1.00	3.00
	Normal saline	8				1.00	1.00
Edema	ACR	8	3.00	2.25	3.00	2.00	3.00
	H1.25+ACR	8	2.00	2.00	2.25	2.00	3.00
	H2.5+ ACR	8	2.00	2.00	3.00	1.00	3.00
	H5+ ACR	8	2.00	1.25	3.00	1.00	3.00
	Vit E+ ACR	8	1.50	1.00	2.00	1.00	2.00
	H5	8	2.00	1.00	2.00	1.00	2.00
	Normal saline	8	1.00	1.00	2.00	1.00	2.00
Ischemic cell changes	ACR	8	3.00	2.00	3.00	2.00	3.00
	H1.25+ACR	8	3.00	2.00	3.00	2.00	3.00
	H2.5+ ACR	8	2.00	2.00	2.00	2.00	3.00
	H5+ ACR	8	2.00	1.00	2.00	1.00	2.00
	Vit E+ ACR	8	1.50	0.75	2.25	.00	3.00
	H5	8	1.00	0.50	2.00	.00	2.00
	Normal saline	8	1.00	0.50	1.00	.00	1.00

Table 3. Description and comparison of cerebellum pathological parameters between the groups.

Pathological index	Group	N	Median	Q1	Q3	Min	Max
Hyperemia	ACR	8	3.00	2.25	3.00	2.00	3.00
	H1.25+ACR	8	3.00	2.75	3.00	2.00	3.00
	H2.5+ ACR	8	3.00	2.00	3.00	2.00	3.00
	H5+ ACR	8	2.00	2.00	3.00	2.00	3.00
	Vit E+ ACR	8	2.00	1.75	2.25	1.00	3.00
	H5	8	2.00	2.00	2.50	2.00	3.00
	Normal saline	8	1.00	1.00	1.50	1.00	2.00
Hemorrhage	ACR	8	3.00	3.00	3.00	3.00	3.00
	H1.25+ACR	8	3.00	2.00	3.00	2.00	3.00
	H2.5+ ACR	8	2.00	2.00	3.00	2.00	3.00
	H5+ ACR	8	2.00	1.25	2.75	1.00	3.00
	Vit E+ ACR	8	1.00	1.00	1.25	1.00	2.00
	H5	8	2.00	1.00	2.00	1.00	2.00
	Normal saline	8	1.00	0.50	1.00	.00	1.00
Ischemic cell changes	ACR	8	3.00	2.25	3.00	2.00	3.00
	H1.25+ACR	8	2.00	2.00	3.00	2.00	3.00
	H2.5+ ACR	8	2.00	2.00	2.00	2.00	3.00
	H5+ ACR	8	2.00	1.25	2.00	1.00	3.00
	Vit E+ ACR	8	1.00	.00	2.00	.00	2.00
	H5	8	1.00	.00	2.00	.00	3.00
	Normal saline	8	1.00	.00	1.00	.00	1.00

The histopathological examinations of rats' brain tissues in the control group revealed a normal histological structure while animals of the ACR-treated group exhibited tissue lesions such as ischemic cell changes, hyperemia, hemorrhage and edema in the cerebrum (Fig. 1); ischemic cell changes, hyperemia and hemorrhage in cerebellum (Fig. 2). Treatment with different doses of honey - especially the dosage of 5 g/kg- considerably reduced the severity of tissue lesions caused by ACR to the extent that in some treated brain tissues, no notable tissue lesions were detectable (Fig. 3 and 4).

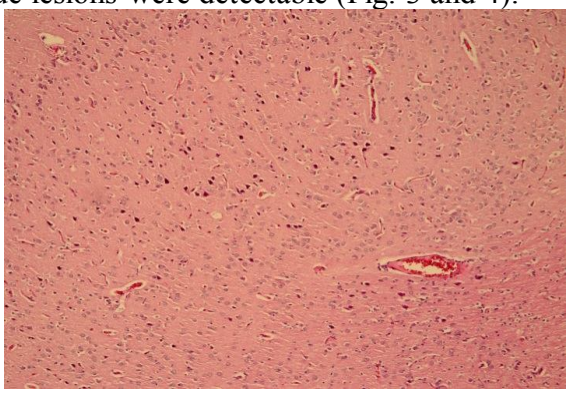

Figure 1. Cerebrum of an ACR-treated rat; ischemic cell changes, hyperemia, and edema (hematoxylin and eosin stain, ×100).

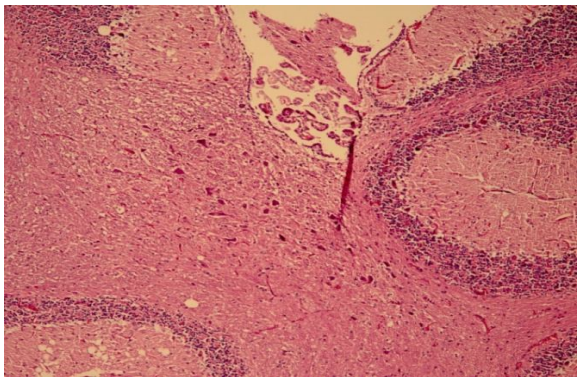

Figure 2. Ischemic cell changes in Cerebellum (hematoxylin and eosin stain, ×100).

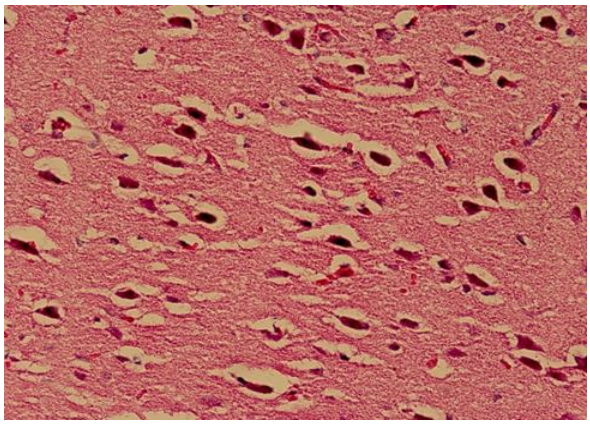

Figure 3. Cerebrum of group 3; ischemic cell changes and edema (hematoxylin and eosin stain, ×400).

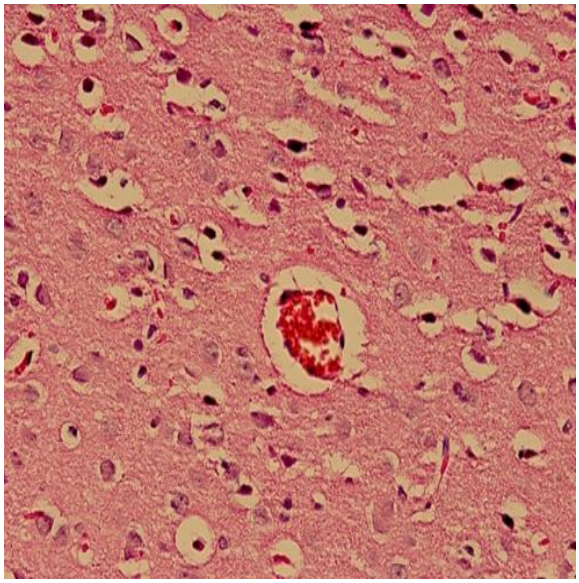

Figure 4. Cerebrum of group 5; hyperemia and edema (hematoxylin and eosin stain, ×400).

The obtained results from the statistical analyses in cerebrum tissue are shown as follows:

Ischemic cell changes in groups 2, 3, 4 and 5 were significantly more than the control group, and in group 2 were significantly more than group 5 (P=0.005). Ischemic cell changes in group 3 were significantly more than group 5 (P=0.008). This factor in group 2 was significantly more than group 6 (P=0.003) and control (P=0.002) group.

Edema in the group 2 was significantly more than groups 1 (P=0.005), 5 (P=0.007), and 6 (P=0.004).

Hemorrhage in groups 2 and 3 (P=0.002), 4 and 5 (P=0.006) was significantly more than control group. Hemorrhage in the group 3 was significantly more than group 6 (P=0.007).

Results obtained from the statistical analyses in cerebellum indicated as follows:

Hemorrhage in the group 2 was significantly more than groups 4 (P=0.005), 5 (P=0.003), 6 (P=0.000) and 7 (P=0.001). Hemorrhage in groups 3 and 4 groups was significantly more than group 6 (P=0.004). This change in groups 2 (P=0.001), 3 (P=0.004) and 4 (P=0.003) was significantly more than control group.

Hyperemia in groups 2 (P=0.003), 3 (P=0.004), 4 and 5 (P=0.006) was significantly more than control group.

Histopathological Assessment of Liver Tissue in Rats and Protective Effect of Honey

Results of histopathological examination and statistical analyses of liver tissues are recorded in Table 4.

Table 4. Description and comparison of liver pathological parameters between the groups.

Pathological index	Group	N	Median	Q1	Q3	Min	Max
Degeneration	ACR	8	3.00	2.25	3.00	2.00	3.00
	H1.25+ACR	8	2.50	2.00	3.00	2.00	3.00
	H2.5+ ACR	8	2.00	2.00	2.75	2.00	3.00
	H5+ ACR	8	1.50	1.00	2.00	1.00	2.00
	Vit E+ ACR	8	1.50	1.00	2.25	1.00	3.00
	H5	8	1.00	1.00	1.50	1.00	2.00
	Normal saline	8	1.00	.00	1.00	.00	1.00
Hemorrhage	ACR	8	2.00	2.00	3.00	2.00	3.00
	H1.25+ACR	8	2.00	2.00	3.00	2.00	3.00
	H2.5+ ACR	8	3.00	2.00	3.00	2.00	3.00
	H5+ ACR	8	2.00	2.00	3.00	2.00	3.00
	Vit E+ ACR	8	2.00	1.75	2.25	1.00	3.00
	H5	8	1.00	1.00	2.50	1.00	3.00
	Normal saline	8	1.00	1.00	1.50	1.00	2.00
Inflammation	ACR	8	3.00	2.00	3.00	2.00	3.00
	H1.25+ACR	8	2.00	2.00	2.00	2.00	2.00
	H2.5+ ACR	8	2.00	2.00	2.75	1.00	3.00
	H5+ ACR	8	2.00	1.25	2.25	1.00	3.00
	Vit E+ ACR	8	2.00	1.00	2.00	1.00	3.00
	H5	8	1.00	1.00	2.00	1.00	3.00
	Normal saline	8	1.00	.00	1.00	.00	1.00
Hyperemia	ACR	8	2.50	2.00	3.00	2.00	3.00
	H1.25+ACR	8	2.00	1.00	2.25	1.00	3.00
	H2.5+ ACR	8	2.00	2.00	2.00	2.00	2.00
	H5+ ACR	8	2.00	2.00	2.75	1.00	3.00
	Vit E+ ACR	8	2.00	2.00	2.00	2.00	2.00
	H5	8	1.00	1.00	2.50	1.00	3.00
	Normal saline	8	1.00	1.00	1.50	1.00	2.00

The histopathological studies of the liver tissue in the control animals showed normal histological features with well-distinguished hepatic lobules, separated by interlobular septa, traversed by portal veins, hepatic artery and bile ducts (hepatic triads). The rats treated with ACR exhibited histopathological alterations that included degeneration of hepatic cells, necrotic changes in the hepatic cells, hyperemia in blood vessels and hepatic sinusoids, hemorrhage and inflammation (Figs. 5 and 6). The rats treated with honey revealed much less injury to liver structure (Figs. 7 and 8). Honey reduced the severity of ACR-induced tissue lesions in rats' liver tissues.

Data of the statistical analyses in liver tissue presented as follows:

Degeneration in the groups 2, 3 and 4 was considerably more than groups 1, 5 and 7.

Inflammation in groups 2 and 3 (P=0.002), 4 (P=0.005), and 5 (P=0.008) was significantly more than control group.

Hemorrhage in groups 2 and 5 (P=0.006), and 4 (P=0.004) was considerably more than control group.

Hyperemia in the groups 2 (P=0.005), 4 (P=0.003) and 6 (P=0.009) was considerably more than control group.

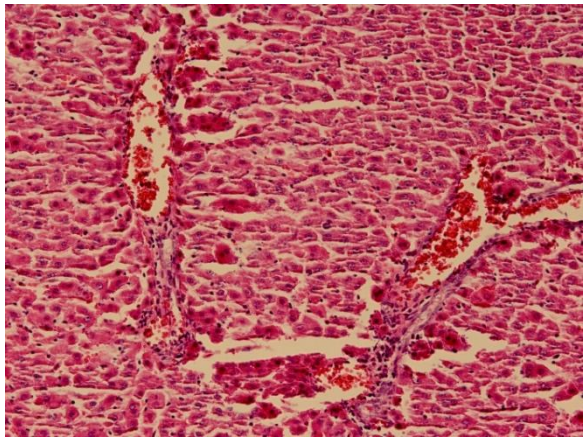

Figure 5. Liver of an ACR-treated rat; degeneration of hepatic cells and hyperemia (hematoxylin and eosin stain, ×400).

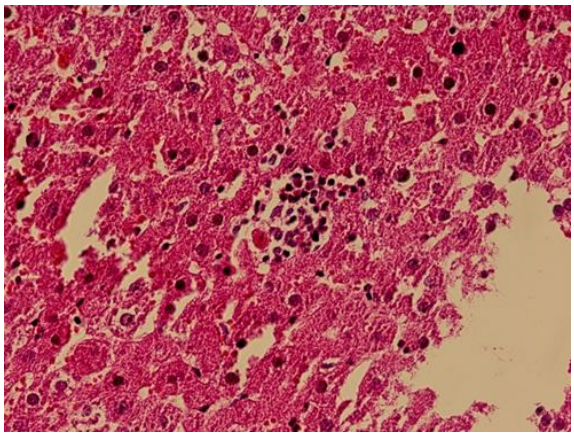

Figure 6. Liver of ACR-treated group; inflammation (hematoxylin and eosin stain, ×400).

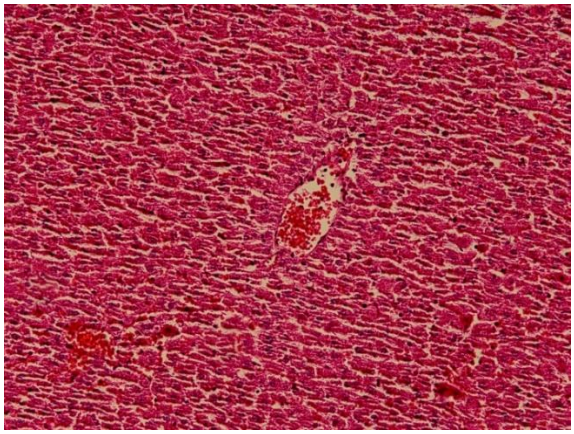

Figure 7. Liver of group 4; degeneration of hepatic cells and hyperemia (hematoxylin and eosin stain, ×200).

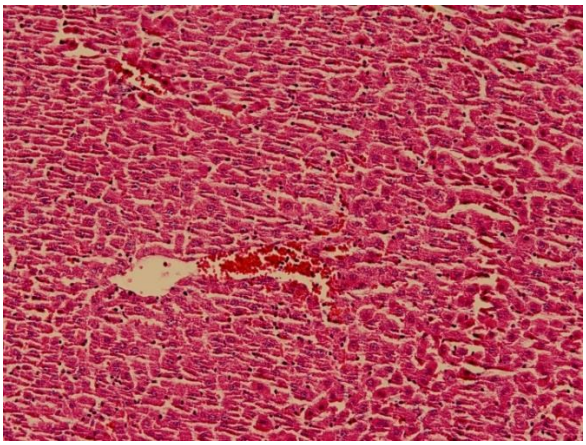

Figure 8. Liver of group 5; hyperemia (hematoxylin and eosin stain, ×200).

DISCUSSION

The present research was designed to consider the protective effects of honey on tissue lesions due to acrylamide subacute toxicity in Wistar rats.

Acrylamide is a toxic chemical compound that causes injuries in the tissues of the brain, lung, liver, intestine, kidney, and testes via oxidative stress and this has been reported by many [22]. Therewith, the toxicity of acrylamide is in a dose-dependent manner [7]. Accordingly, many researchers studied various protective compounds against ACR- induced toxicity and recently acceptable results have been attained by using many natural compounds [15].

The results of this study revealed that administration of ACR (50 mg/kg IP for 11 d) resulted in histopathological changes such as degeneration of hepatic cells, necrotic changes in the hepatic cells, hyperemia in blood vessels and hepatic sinusoids, hemorrhage and inflammation in liver tissues. Our results agreed with other studies [7, 13, 23-25]. These tissue injuries have resulted from generation of reactive oxygen species (ROS) by ACR which elevated lipid peroxidase production [7] and subsequently caused increase in the lipid peroxidation, protein carbonyl, lipid peroxyl radicals and hydroperoxide levels [7,14]. In addition, significant increase in malondialdehyde (MDA) level was observed. MDA was used as a marker for oxidative stress of protein and lipoproteins. Exposure to ACR markedly decreases liver cellular glutathione (GSH) level [7, 23]. GSH is an important antioxidant that prevents damage to cells and maintains cell integrity against obtained exogenous and endogenous ROS [26]. Conjugation of ACR and glycidamide MDA uses as a marker for oxidative stress of protein and lipoproteins with glutathione is the most acceptable reason for the decrease of GSH [24]. ACR leads to induced oxidative stress that injures the membranous system of hepatocytes. Moreover, ACR makes interference in the liver enzymes for example glutathione S-transferase (GST) and interference in these enzymes can lead to tissue lesions [24]. In addition, affinity of ACR for sulfhydryl groups on proteins has been confirmed [27]. Therefore, histopathological lesions observed in the liver of the ACR-treated group could be due to the reaction of acrylamide and glycidamide with liver proteins. These are possible mechanisms for liver damage.

Histopathological alterations of ACR recipient rats with a dose of 50 mg/kg in the current study revealed ischemic cell change, hyperemia, hemorrhage and edema in cerebrum; ischemic cell change, hyperemia, and hemorrhage in cerebellum tissue. These results are similar to other reported [13, 15, 21 and 26].

An imbalance between the creation and elimination of free radicals and ROS increases oxidative stress related to the pathogenesis of some neurodegenerative conditions [14, 26]. Thus, the pathological mechanisms of acrylamide-induced

neurotoxicity associated with depletion of antioxidants and reduction in glutathione, enhancement in the production of ROS, increment levels of lipid peroxidative (LPO) products, detracting in the activities of enzymatic and non-enzymatic antioxidants with decline in acetylcholinesterase (AChE) activity in brain where acetylcholine is an important (substantial-main) neurotransmitter and its function is related to its metabolizing enzyme AChE. Production of free radicals by ACR can inhibit the activity of enzyme AChE. Thus, inhibition of AChE could result in disorder in the metabolic and nervous activity [14].

Reduction in the level of glutathione (GSH) in ACR toxicity leads to a weakening in the body antioxidant defense system against free radicals induced injury. It also leads to an increase in the vulnerability of cell membrane and cellular damage and it will eventually lead to neurodegenerative lesions in the brain [14]. Acrylamide induces the oxidative stress associated with enhancement of neuronal intracellular calcium ion concentration along with neurodegeneration [28].

Moreover, these may be ascribed to the fact that acrylamide and/or glycidamide binds to dopamine receptors and inhibits the activity of motor proteins (e.g kinesin and dynein) leading to interference with neural intracellular transport [7]. In addition, the terminal nerves are the primary site of acrylamide action and inhibition of neurotransmission intercedes the extension of neurological deficits or disrupts presynaptic nitric oxide (NO)signaling [7].

Since the ACR can increase the generation of ROS and the potential role of oxidative stress in the pathogenesis of tissue lesions, antioxidant compounds can be considered as an alternative therapeutic approach in diminishing ACR toxicity [14, 26].

Honey antioxidant properties are related to the existence of specified compounds such as polyphenols which may ameliorate oxidative stress in various organs [20]. The antioxidant effects of honey in various conditions expressed in many studies. Protective action of honey against the histological changes and oxidative stresses induced by cigarette smoke in rat's testis by reduction lipid peroxidation and restoring the antioxidant system in rat's testis out of reducing oxidative stress [19]. Honey can protect against oxidative stress and histopathological alteration induced by monosod-ium glutamate by its antioxidant activity [16]. The favorable hepato-nephro-protective effects of honey were reported against acetaminophen-induced oxidative damage in Wistar rats and this finding was confirmed biochemically and histopathologically [29]. Therefore, honey has a preservative effect against tissue injuries and oxidative stress caused by ACR in rat tissues through its antioxidant property.

In the present research, oral administration of honey with different doses -particularly the high dose (5 g/kg)- remarkably reduced the severity of tissue lesions induced by ACR compared with ACR receiver rats and moderated the ACR induced pathological damages and oxidative stress in rat brain and liver.

CONCLUSION

The severity of tissue lesions caused by the ACR can be reduced by honey, likely through its antioxidant activity, and it ameliorated ACR induced oxidative stress in rat tissues. Therefore, honey probably can be used to protect tissues against histopathological lesions and oxidative stress caused by ACR. Honey effectiveness increased with its concentration enhancement.

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