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Neurophysiological Study on Possible Protective Effect of Rosemary (*Rosemarinus officinalis*) Leaves Extract in Male Albino Rats Treated with Acrylamide

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Abstract: Rosemary (*Rosmarinus Officinalis* L) leaves extracts show a very high antioxidant activity and increasingly used as food additives, proposed as important human dietary factor.In this study, the neuroprotective effect of The Rosemary (*Rosmarinus officinalis* L) leave extract was investigated against acrylamide-induced neurotoxicity in male albino rats. The daily dose (30mg/kg b.w.) i.p. injection of pure acrylamide (ACR) for four weeks caused a significant decrease in catecholamine: epinephrine (E), norepinephrine (NE) and dopamine (DA) content all tested areas (cerebellum, brainstem, striatum, cerebral cortex, hypothalamus and hippocampus) at most of the time intervals studied. This is may be due to axon and nerve terminal degeneration, which caused changes in transmitter synthesis, storage uptake, release and reduction in synaptic vesicle as a result the content of neurotransmitters is decreased. whereas, daily dose (100mg/kg b.w.) i.p. injection of rosemary extract for 30 days and subsequent withdrawal caused a significant decreased in catecholamine content all tested areas at most of the time intervals studied. This is may be, in part due to the presence of caffeic acid and rosmarinic acid which affected the uptake of monoamines and monoamines oxidase activity. At the same time the extract contain ursolic acid (UA) and carnosol which increase in nitric oxide (NO) levels so the content of catecholamine is decreased. The present study revealed that moderate improvement in catecholamine content alteration caused by acrylamide, this is may be due to its attributed to its antioxidant and free radical scavenging activities. In conclusion. the results suggest that Rosemary (*Rosmarinus Officinalis* L) leaves extract neuroprotective effects against ACR-induced neurotoxicity injury.

Key words: Rosemary % Acrylamide % *Rosemarinus officinalis* % E % NE % DA % Brain

INTRODUCTION

In 1989, Collins *et al*. reported the mortality experience of 8854 workers with potential exposure to acrylamide (ACR), a substance widely used in the manufacture of water soluble polymers used for water treating, paper mining and sugar processing, at four Cytec Industries (formerly the chemical division of American Cyanamid Company). Recently high levels were unexpectedly detected in widely consumed food items, notably French fries, potato crisps, bread [1]. Much international public concern arose since acrylamide has been classified as a probable carcinogen [2]. A few month later, reports from laboratory studies have provided insight into the biochemical mechanism of acrylamide formation. Acrylamide can be generated during the heating of specific foodstuffs as a result of Maillared reaction between amino acids and sugar [3,4].

French fries and potato chips are common parts of children's menus fast in fast food restaurant, over the past 30 years ; these familiar food contain high levels of toxic and carcinogenic byproducts not found in the uncooked foods [5,6].

The classification of a acrylamide by International Agency for research on Cancer (IARC) 1994, as a probable human carcinogen, was mainly based on in Vitro and animal models. Acrylamide induces genetic mutation and chromosomal abnormalities in vitro and cellular transformation in vivo [7]. Long-term studies in rats and mice supported a dose exposure relation between acrylamide and risk of cancer of the lung, mammary gland, thyroid gland, lining of oral activity and intestinal and

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reproductive tracts [8]. Moreover, animals administration acrylamide orally or fed a diet high in fried foods [9] had higher levels of hemoglobin DAN adduct compared to unexposed animals. It has been demonstrated that reproductive toxicity is not only induced by ACR, but also by its metabolite glycidamide (GA) [10,11].

Plant material in the human diet contains a large number of natural compounds, which may be of benefit in protecting the body. one of the plants with constituents reputed to possess antioxidant properties was rosemary.

Rosemary (*Rosmarinus Officinalis* L), an evergreen shrub, is one of the herb spices of the family Labiatae. It was cultivated in Mediterranean first, then transplanted to China in Dynasty, but cultivated in all of the world now [12].

Leaves of R. Officinalis possess a variety of bioactivities, including antioxidant, antitumor, anti-inflammatory, treat headaches and anti-HIV [13,14].Extract of Rosmarinus Officinalis L leaves contains flavonoids, phenols, volatile oil and terpenoids [15,16].

Consequently, the present studies has been carried out in an attempt to evaluate the role of Rosemary (Rosmarinus Officinalis L) leaves extract as a protective agent against acrylamide neurotoxicity, by evaluating the changes in catecholamine: epinephrine (E), norepinephrine (NE) and dopamine (DA) content in different brain region (cerebellum, brain stem, striatum, cerebral cortex, hypothalamus and hippocampus), since these neurotransmitters are known to play an important role in brain function.

MATERIALS AND METHODS

Chemicals and Plant Extract: Acrylamide was purchased from Sigma as a white powder. Fresh rosemary (*Rosmarinus Officinalis* L) was collected from Tabuk, Saudi Arabia between the month of May and July 2008. Rosemary was extracted according to the method described by [17]. The plant was dried under shade at 25C° and the dried leaves of plant were grounded with a blender. The powder part was kept in nylon bags in deep freezer until the time of use.

Animals: The experimental animals used in this study were adult male albino rats, *Rattus rattus* with an average body weight 100-120 g. Animals were reared in animal house at the center of king Fahad of medical researchers in Jeddah, Saudi Arabia. The animals were supplied with food and water *ad libitum* under standard conditions of light, humidity and temperature. The treated animals were randomly divided into four group. The first group (n=24) was divided into four subgroups each of 6 rats. The animals were daily injected (i.p.) with 30 mg/kg of acrylamide [18,19] and one subgroup was decapitated after 1, 2, 3 and 4 weeks. The second group (n=24) was divided as the first group but the rats were injected (i.p.) with 100 mg/kg of rosemary (*Rosmarinus Officinalis* L) leaves extract [20]. The 3rd group(n=6) was injected daily with rosemary (*Rosmarinus Officinalis* L) leaves extract (100 mg/kg i.p.) for 3 weeks then injected with acrylamide (30 mg/kg i.p.) for one week, the rats were decapitated after 4 weeks. The 4th group(n=24) divided as the first group, the rats were injected with saline vehicle and served as control.

The treated rats were decapitated at the designed time intervals. The brain was rapidly and carefully excised, separated into 2 halves. Each half was then dissected on dry ice glass plate, according to the method of Glowinski and Iversen [21], into the following regions: cerebellum, brain stem, striatum, cerebral cortex, hypothalamus and hippocampus. The brain tissues were wiped dry with a filter paper, weighed, wrapped in plastic films and then in aluminum foil and quickly frozen in dry ice pending analysis.

E, NE and DA and were extracted and estimated according to the method of Chang modified by Ciarlone [22,23]. The fluorescence was measured in Jenway 6200 fluorometer.

Statistical Analysis: All the values obtained from animals are revealed as mean + S.E. Data from 6 animals each for experimental and control groups were analyzed using Student's ''t''-test and results were considered significant at $P < 0.01$ [24]. All statistical analysis were computed by SPSS version 14. Percentage difference is representing the percent of variation with respect to the control.

RESULTS

The single daily i.p. injection of 30 mg/kg of acrylamide significantly decreased the E content in all tested areas (Figure 1). The maximal decrease was found in hypothalamus after 4 weeks.

Data in Figure 2 show that the treatment induced a significant decrease in NE content in all tested areas. The maximal decrease was found in hypothalamus after 4 weeks.

Figure 3 shows that 30 mg/kg (i.p.) of acrylamide caused a significant decrease in DA content in all tested areas. The maximal decrease was found in brain stem after 4 weeks.

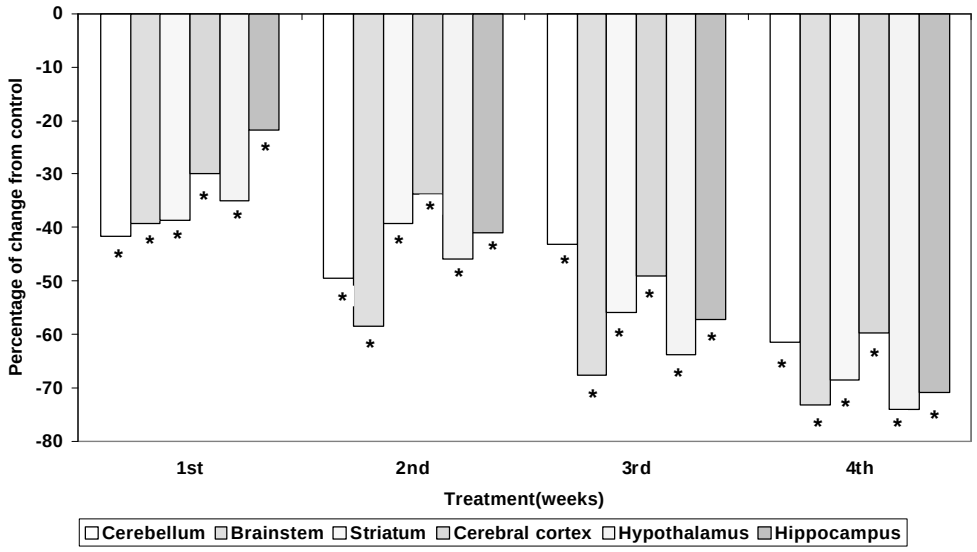

Fig. 1: Effect of chronic administration of acrylamide (30mg/kg, i.p.) on epinephrine (E) content (µ g/g) represented by the % different between control and treated values in different brain areas of male albino rat.
* significant at p <0.01

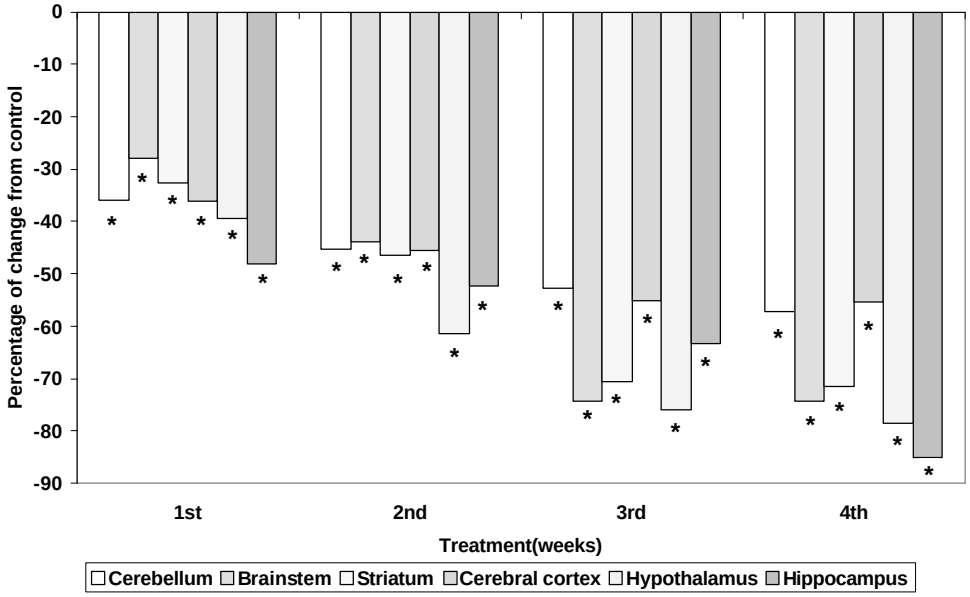

Fig. 2: Effect of chronic administration of acrylamide (30mg/kg, i.p.) on norepinephrine (NE) content (µ g/g) represented by the % different between control and treated values in different brain areas of male albino rat.
* significant at p <0.01

Figure 4 shows that the daily i.p. administration of rosemary (*Rosmarinus Officinalis* L) leaves extract (100mg/kg b.wt.) for 4 weeks caused a significant decrease in epinephrine (E) content in all areas after 1 and 2 weeks except in hippocampus after 1 week and striatum after 2 weeks. The E content still significantly decreased in brain stem after 3 and 4 weeks and in hypothalamus after 3 weeks. The maximal decrease was found in brain stem after 3 weeks.

As shown in Figure 5, rosemary extract (100mg/kg b.wt.) caused a significant decreased in NE content in all tested areas after one week except in cerebral cortex.

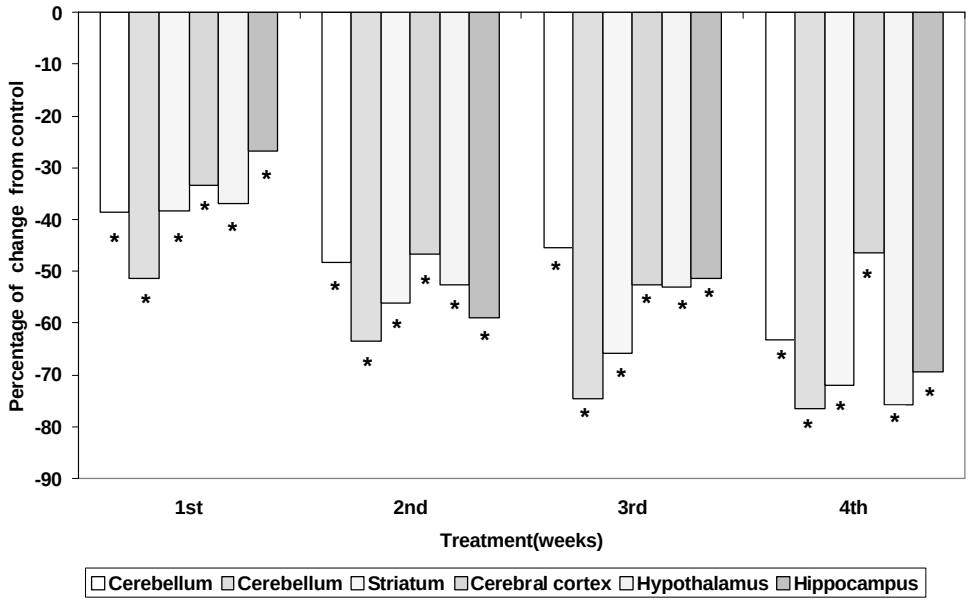

Fig. 3: Effect of chronic administration of acrylamide (30mg/kg, i.p.) on dopamine (DA) content (µ g/g) represented by the % different between control and treated values in different brain areas of male albino rat.
* significant at p <0.01

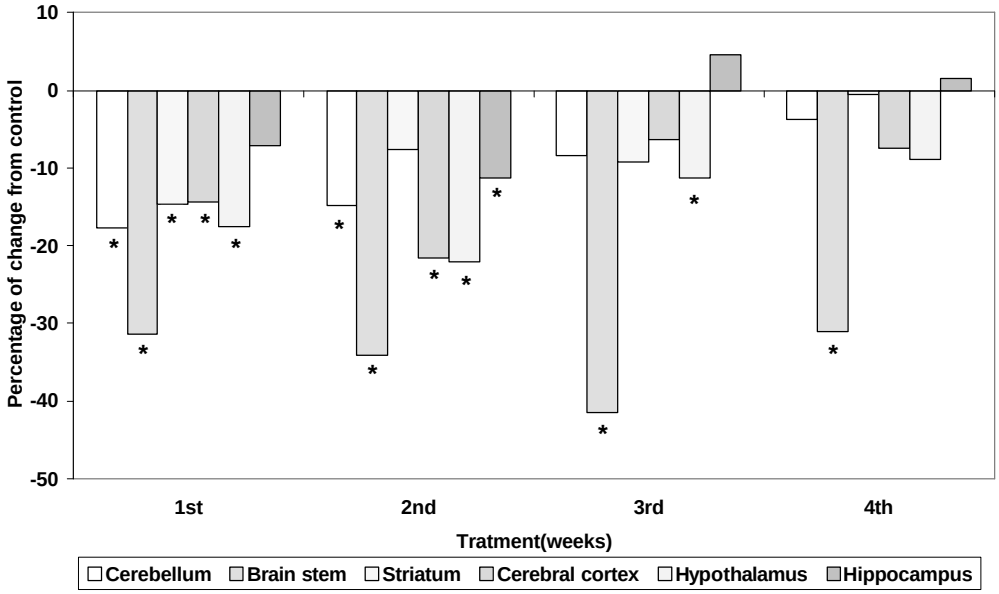

Fig. 4: Effect of daily administration of rosemary (*Rosmarinus Officinalis* L) leave extract (100mg/kg, i.p.) on epinephrine (E)content (µ g/g) represented by the % different between control and treated values in different brain areas of male albino rat.
* significant at p <0.01

The NE content still significantly decreased in cerebellum, brain stem and hypothalamus after 2 weeks. After 3 and 4 weeks NE content returned to the normal level in all tested areas except in brain stem. The maximal decrease was found in brain stem after one weeks.

Figure 6 shows that daily administration of rosemary extract induced a significant decreased in DA content in all tested areas after 1,2 and 3 weeks except in: Striatum after 1,2 and 3 weeks, in cerebral cortex after 2 weeks and in hypothalamus after 3 weeks. The DA content still

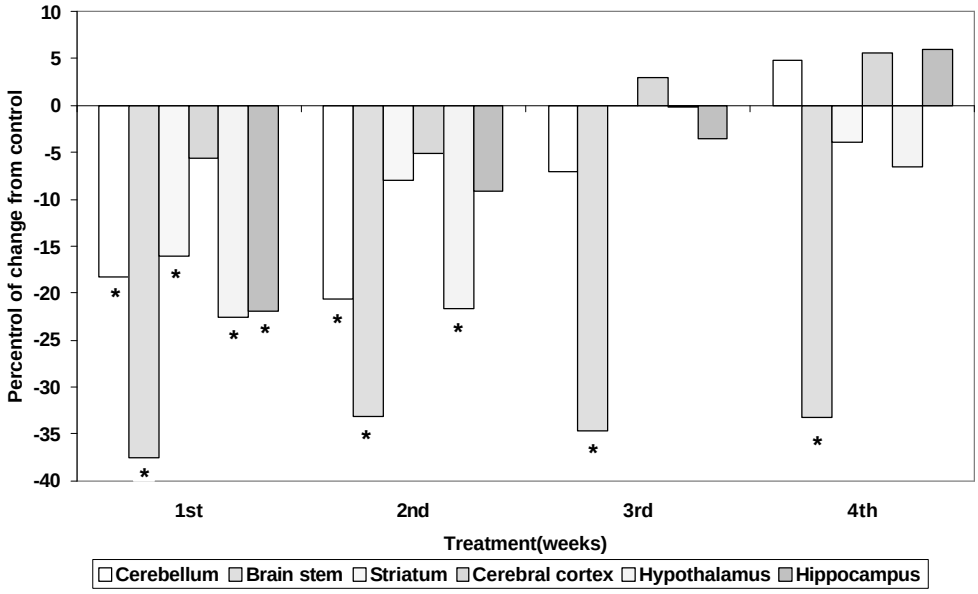

Fig. 5: Effect of daily administration of rosemary (*Rosmarinus Officinalis* L) leave extract (100mg/kg, i.p.) on norepinephrine (NE) content (µ g/g) represented by the % different between control and treated values in different brain areas of male albino rat.
* significant at p <0.01

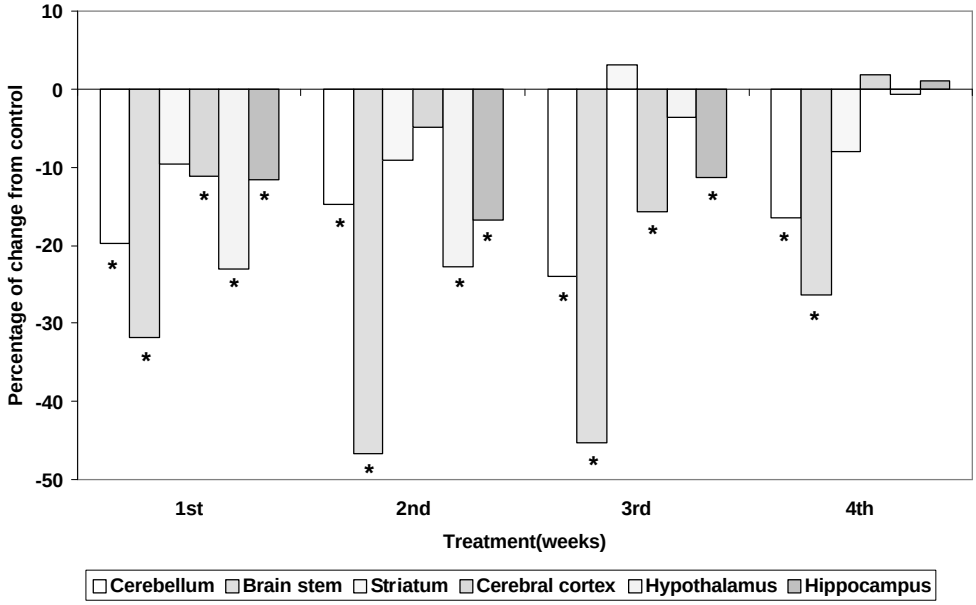

Fig. 6: Effect of daily administration of rosemary (*Rosmarinus Officinalis* L) leave extract (100mg/kg, i.p.) on dopamine (DA) content (µ g/g) represented by the % different between control and treated values in different brain areas of male albino rat.
* significant at p <0.01

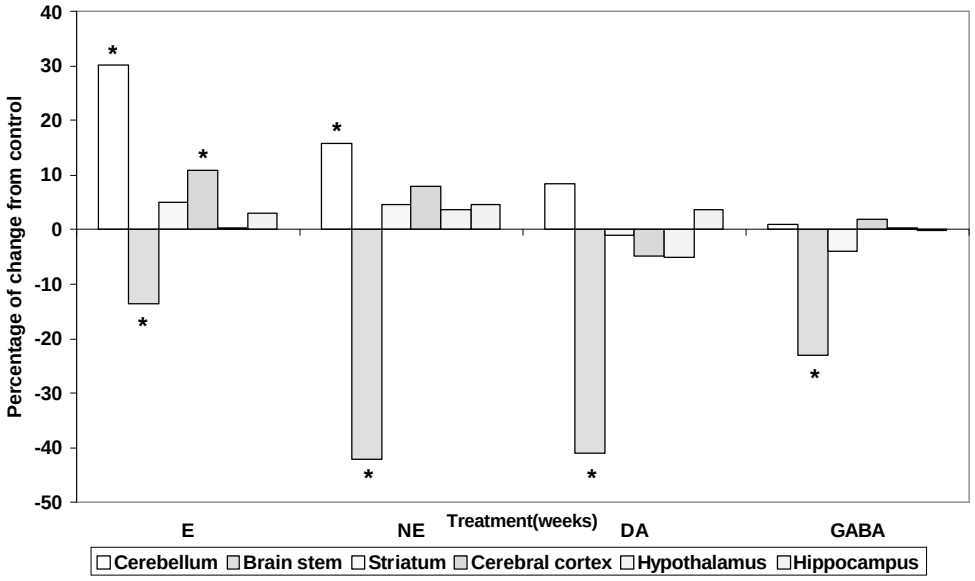

Fig. 7: Effect of chronic administration of Rosemary (*rosmarinus officinallis*) leave extract (100mg/kg, i.p.) for 3 weeks and acrylamide (30mg/kg, i.p.) for one week on epinephrine (E), norepinephrine (NE), dopamine (DA) content (µ g/g) represented by the % different between control and treated values in different brain areas of male albino rat.
* significant at p <0.01.

significantly decreased after 4 weeks in cerebellum and brain stem. The maximal decrease was found in brain stem after 2 weeks.

Figure 7 shows that administration of acrylamide (30mg/kg, i.p.) for one week after treatment with rosemary (*Rosmarinus Officinalis L*) leaves extract (100mg/kg, i.p.) for 3 weeks caused a significant increased in E content in cerebellum and cerebral cortex, whereas there was a significant decrease in brain stem and insignificant changes in the other tested brain areas. The NE content significantly increased in cerebellum and significantly decreased in brain stem and insignificant changes in the other tested brain areas. There was a significant decreased in DA content in brain stem and insignificant changes in the other tested brain areas.

DISCUSSION

The previous studies were carried by Li *et al* and Totni *et al*, demonstrated that acrylamide is a well do cemented neurotoxicant in both human and laboratory animal [25,26]. It has been demonstrated that reproductive toxicity is not only induced by acrylamide, but also by glycidamide [27].In 1981, Goldstien and Lowndes suggested that, defective neurotransmission in acrylamide- intoxicated laboratory animals might be mediated by changes in transmitter synthesis, storage, uptake and release [28]. Nerve terminal dysfunction in thalamus, basal ganglia and other brain regions might play a role in the sensory, autonomic and motor deficits that are characteristic of ACR neurotoxicity [29-31].

Results from the present study show that intoxication of rats at the higher dose –rate (30mg/kg per day) produced significant decreased in epinephrine, norepinephrine, dopamine and gamma-aminobutyric acid contents in all tested brain areas at the different time intervals.

Goldstein and Lowndes and Sickles *et al*., suggested that defective neurotransmission in ACR- intoxicated laboratory animal might be mediated by changes in transmitter synthesis, storage uptake and release [28, 32]. Ali and Aldous *et al*. cited that, administration of 10 and 20 mg/kg body weight i.p. of ACR in male rat induced changes in the levels of monoamines and their metabolites [33,34]. LoPachin *et al*. suggested that, ACR impaired neurotransmitter uptake into striatal synaptic vesicle[35].

From the present results and the previous studies it could be concluded that, the daily administration of ACR caused a significant decreased in E, NE and DA content in all tested brain areas ; this may be due to ; axon and nerve terminal degeneration, which caused changes in transmitter synthesis, storage uptake, release and

reduction in synaptic vesicle as a result the content of neurotransmitters is decreased.

The previous studies which were carried by Burnett *et al*. and Peng *et al* demonistrated that the reosemary extract is used in folk medicine for improvement of memory and stimulant [36, 17].

Rosemary (*Rosmarinus Officinalis* L) leaves extracts show a very high antioxidant activity and increasingly used as food additives, proposed as important human dietary factor [37,38]. Rosemary contain flavonoids, phenols, volatile oil and terpenoids: carnosic acid, carnosol, rosmanol, caffeic acid and urosolic acid [39-41]. The most important constituent of rosemary are caffeic acid and its derivative, rosmarinic acid [42]. Tsuji *et al*. suggested that caffeic acid and rosmarinic acid affected the uptake of monoamines and monoamines oxidase activity [43]. Sozio *et al*. demonstrated that caffeic acid and carnosine improve dopamine release in the brain[44].

From the present results it is clear that the administration of rosemary (*Rosmarinus Officinalis* L) leaves extract caused a significant decreased in E, NE. and DA content in most of tested brain areas at different time intervals.

You *et al* found that Ursolic acid (UA) elicited a dose-dependent increase in nitric oxide (NO) and TNF-alpha production and the level of nitric oxide synthase (iNOS) and TNF-alpha mRNA[45]. Kellner and Zunino cited that intracellular nitric oxide levels increased significantly after exposure to the antioxidants curcumin, carnosol and quercetin [46]. Nitric oxide (NO) is a short-lived small molecule free redical produced from L-arginie, It has many biological functions involved in vasodilation, neurotransmission and tissue homeostasis [41]. NO formed by the activation on N-methyl-D-aspartate (NMDA) receptors. NMDA receptors stimulation is known to depolarize neuronal membrane and consequently to increase CA^{2+} influx into neurons in association with opening of voltage-dependent CA^{2+} channels. The increase in CA^{2+} influx is assumed to contribute to the initation of CA^{2+} dependent exocytotic release of [47,48]. Direct application of NO generators to preparations from the brain also release [49,50].

From the Present results and the previous studies it could be concluded that the decrease in the neurotransmitter (E, NE, DA) content in tested brain areas after the administration of rosemary extract may be, in part due to the presence of caffeic acid and rosmarinic acid which affected the uptake of monoamines and monoamines oxidase activity. At the same time the extract contain ursolic acid (UA) and carnosol which increase in nitric oxide (NO) levels so the content of catecholamine is decreased.

Several lines of evidence indicated that the neuroprotective effects of carnosic acid critically required both free carboxylic acid and catechol hydroxyl moictics [51]. The rosemary extract also demonstrated higher DPPH (2,2'-diphenyl-1-picrylhydrazyl) free radical scavenging capability [52]. In *vivo* studies have shown that the diterpene carnosic acide may protect biological membrane from oxidative dameage [53]. Park *et al* reported that carnosic acide safeguard dopaminergic neuronal cells from environmental neurotoxins [54].

The Protective effect of the rosemary extract against acrylamide toxicity was clear in the present study.The extract improve the content of catecholamine content in most of tested brain areas compared with animal group that received monosodium glutamate.

In conclusion, the present study showed that the neurochemical damage of the brain areas, caused a decreased in catecholamine content under the effect of acrylamide can be minimized by rosemary extract which improve content of catecholamine in most of deferent brain areas,this is may be due to its attributed to its antioxidant and free radical scavenging activities.

REFERENCE

1. WHO 2002. FAO/WHO consultation on the health implication of acrylamide in food. Summary report of a meeting held in Geneva, Switzerland, Jun 25-27.
2. Mucci, L.A., P.W. Dickman, G. Steincck, H.O. Adami and K. Augustsson, 2003. Dietary acrylamide and cancer of the large bowel, kidney and bladedr: Absence of an association in a population-based study in Sweden. Br. J. Cancer, 88: 84-89.
3. Mottram, D.S., B.L. Wedzicha and A.T. Dodson, 2002. Acrylamide is formed in the Millared reaction.Nature., 419: 448-449.
4. Stadler, R.H., I. Blank, N. Varga, F. Robert, J. Hau, P.A. Guy, M.C. Robert and S. Riediker, 2002. Acrylamide from Millared reaction product. Nature, 419: 448-449.
5. Mitka, M., 2002. Fear of frying: Is acrylamide in foods a cancer risk ? JAMA, 288(17): 2105-2106.
6. Madbouly, S.M., 2006. Response of the intestinal mucosa of rats to acrylamide administration and the possible prophylactic role of garlic oil: histological and ultrastracturel studies (transmission and scanny electron microscope). Histology & Histochemistry, 50(C): 161-182.
7. IARC 1994. Monographs on the evaluation of carcinogen risk to humans: Some industrial chemicals. No. 60, Lyan: International Agency for Research on Cancer, 60: 389-433.

8. Johnson, K.A., S.J. Gorzinski, K.M. Bodner, R.A. Campbell, C.H. Wolf, M.A. Friedman, *et al*. 1986. Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. Toxicol. Appl. pharmacol., 85(2): 154-168.
9. Tarek, E., P. Rydberg, P. Karlsson, S. Eriksson and M. Tornquist, 2000. Acrylamide: A cooking carcinogen ?Chem. Res. Toxicol., 13: 517-522.
10. Adler, I.D., A. Baumgartner, H. Gonda, M.A. Friedman and M. Skerhut, 2000. 1-Aminobenzotriazol inhibits acrylamide – induced dominant lethal effects in spermatide of male mice. Mutagenesis, 15: 133-136.
11. Doerge, D.R., G.G. Da Costa, L.P. McDaniel, M.I. Churchwell, N.C. Twaddle and F.A. Beland, 2007. DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. Mutat. Res., 580(1-2): 131-141.
12. Hui-Hur, Z., T.U. Peng-Fei, Z. Kan, W. Hui, W. Bao-Huai and L.U. Jing-Feng, 2001. Antioxidant properties of phenolic diterpenes from Rosmarinus Officinalis. Acta. Pharmacol. Sin., 22(12): 1094-1098.
13. Aruoma, O.I., J.P. Spencer, R. Rossi, R. Aeschbach, A. Khan, N. Mahmood, A. Munoz, A. Murcia, J. Butler and B. Halliwell, 1996. An evaluation of the antioxidant and antiviral action of extracts of rosemary and provencal herbs. Food Chem. Toxicol., 34: 449-456.
14. Altinier, G., S. Sosa, R.P. Aquino, T. Mencherini, R. Della Loggia and A. Tubaro, 2007. Characterization of topical anti-inflammatory compounds in Rosmarinus Officinalis L.J. Agric. Food Chem., 55: 1718-1723.
15. Asada, K., 1999. The water-water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons.Annu. Rev. Plant. Physiol. Plant. Mol. Biol., 50: 601-639.
16. Almela, L., B. Sanchez-Munoz, J.A. Fernandez-Lopez, M.J. Roca and V. Rabe, 2006. Liquid chromatographic-mass spectrometric analysis of phenolics and free radical scavenging activity of rosemary extract from different raw material. J. Chromatogr., 1120: 221-229.
17. Peng, C., J. Su, C. Chyau, T. Sung, S. Ho, C. Peng, and R.Y. Peng, 2007. Supercritical fluid extract of rosemary leaves exhibit potent anti- inflammation and anti-tumor effects. Biosci. Biotechnol. Biochem., 71(9): 2223-2232.
18. Goldstein, B.D., 1985. Acrylamide neurotoxicity: altered spinal mono synaptic responses to quipazine,a serotonin agonist, in cats. Toxicol. Appl. pharmacol., 78(3): 436-444.
19. Jones, H.B. and J.B. Cavanagh, 1984. The evolution of intracellular responses to acrylamide in rat spinal ganglion neurons. Neuropathol. Appl. Neurobiol., 10(2): 101-121.
20. Bakirel, T., U. Bakirel, O.U. Keles, S.G. Ulgen and H. Yardibi, 2007. *In vivo* assessment if antidiabetic and antioxidant activities of rosemary (Rosmarinu officinalis) in alloxan-diabetic rabbits. J. Ethnopharmacol., 4871: 1-10.
21. Glowinski, J. and L.L. Iversen, 1966. Regional studies of catecholamines in the rat brain. I. The disposition of [3H] dopamine and [3H] dopa in various regions of the brain. J. Neurochem., 13: 655-669.
22. Chang, C.C., 1964. A sensitive method for spectrofluorometric assay of catecholamines. Int. J. Neuropharmacol., 4: 643 -649.
23. Ciarlone, A.E., 1978. Further modification of a fluoremetric method for analyzing brain amines. microchem. J., 23: 9-12.
24. Woolson, R.F., 1987. Statisical Methods for the Analysis of Biomedical and Mathematical statistics, John Wiley and Sons Inc., New York, pp: 316.
25. Li,S.X., N. Cui, C.L. Zhang, X.L. Zhao, S.F. Yu and K.Q. Xie, 2006. Effect of subchronic exposure to acrylamide induced on the expression of bcl-2, bax and caspase -3 in the rat nervous system. Toxicology, 217(1): 46-53.
26. Totani, N., M. Yawata, Y. Ojiri and Y. Fujioka, 2007. Effect of trace acrylamide intake in Wister rats.Occup. Environ. Med., 64(6): 396-401.
27. Paulsson, B., N. Kotova, J. Grawe, A. Henderson, F. Granath, B. Golding and M. Tornquist, 2002. Induction of micronuclei in mouse and rat by glycidamide the genotoxic metabolite of acrylamide. Mutat. Res., 516: 101-111.
28. Goldstein, B.D. and H.E. Lowndes, 1981. Group Ia primary afferent terminal defect in cats with acrylamide neuropathy. Neurotoxicollogy, 2: 297-312.
29. Lehning, E.J., C.D. Balaban, J.F. Ross and R.M. Lopachin, 2003. Acrylamide neuropathy.I. Spatiotemporal characteristics of nerve cell damage in forbrain. Neuro Toxicology, 24: 125-136.
30. LoPachin, R.M., 2004. The changing view of acrylamide neurotoxicity. Neurotoxicology, 25: 617-630.
31. LoPachin, R.M., 2005. Acrylamide neurotoxicity: neurological, morphological and molecular end points in animal models. Adv. Exp. Med.Biol., 561: 21-37.
32. Sickls, D.W., S.T. Brady, A. Testino, M.A. Friedman and R.W. Wrenn, 1996. Direct effect of nrurotoxicant acrylamide on kinesin-based microtubule motility. J. Neurosci. Rev., 46: 7-17.

33. Ali, S.F., 1983. Acrylamide-induced changes in the monoamines and their acid metabolites in defferent region of the rat brain. Toxicol. Lett., 17(1-2): 101-105.
34. Aldous, C.N., C.H. Farr and R.P. Sharma, 1983. Evaluation of acrylamide treatment on levels of major brain biogenic amines, their turnover rates and metabolites. Fundam. Appl. Toxicol., 3(3): 182-186.
35. LoPachin, R., D. Barber, D. He and S. Das, 2006. Acrylamide inhibits Dopamine uptake in rat striatal synaptic vesicles. Toxicological Sci., 89(1): 224-234.
36. Burnett, K.M., L.A. Solterbeck and C.M. Strapp, 2004. Scent and mood state following an anxiety–provoking task. Psychol. Rep., 95(2): 707-722.
37. Schwarz, K., W. Ternes and E. Schmauderer, 1992. Antioxidant onstituents of Rosmarinus Officinalis: ? Stability of phenolic diterpenes of rosemary extracts under thermal stress as required for technological processes. Z. Lebens Unter Fors., 195: 104-107.
38. Huang, M., C. Ho, Z.Y. Wang, T. Ferraro, Y. Lou, K. Stauber, W. Ma, C. Georgiadis, J.D. Laskin and A.H. Conney, 1994. Inhibition of skin tumorgenesis by rosemary and its constituents carnosol and ursolic acid. Cancer Res., 54: 701-708.
39. Collin, M.A. and H.P. Charles, 1987. Antimicrobial activity of carnosol and ursolic acid: two anti-oxidant constituents of Rosmarinu officinalis. J. Food Microbiol., 4: 311-315.
40. Abe, F., T. Yamauchi, T. Nagao, J. Kinjo, H. Okabe, H. Higo and H. Akahane, 2002. Urosolic acid as a trypanocidal constituent in rosemary. Biol. Pharma. Bull., 25(11): 1485-1457.
41. Lo, A.H., Y.C. Liang, S.Y. Lin-Shiau, C.T. Ho and J.K. Lin, 2002. Carnosol, an antioxidant in rosemary suppresses inducible nitric oxide synthase through down- regulating nuclear factor-kappaB in mouse macrophages. Carcinogenesis, 23: 983-991.
42. Al-Sereiti, M.R., K.M. Abu-Amer and P. Sen, 1999. Pharmacology of rosemary (Rosmarinus Officinalis Linn.) and its therapeutic potentials. Indian. J. Exp. Biol., 37(2): 124-130.
43. Tsuji, M., K. Miyagawa, T. Takeuchi and H. Takeda, 2008. Pharmacological characterization and mechanisms of novel antidepressive and/or anxiolytic-like substances identified from Perilla Herba. Nihon Shinkei Seishin Yakurigaku Zasshi., 28(4): 159-167.
44. SoZio, P., A. Idnnitelli, L.S. Cerasa, I. Cacciator, C. Cornacchia, F. Cantalamessa and A. Di Stefano, 2008. New L-dopa codrugs as potential anti-Parkinson agents. Arch. Pharm (weinheim)., 341(7): 412-417.s
45. You, H.J., C.Y. Choi, J.Y. Kim, S.J. Park, K.S. Hahm and H.G. Jeong, 2001. Ursolic acid enhances nitric oxide and tumor necrosis factor-alpha production via nuclear factor-kappaB activation in the resting macrophages. FEBS Lett., 509(2): 156-160.
46. Kellner, C. and S.J. Zunino, 2004. Nitric oxide is synthesized in acute leukemia cells after exposure to phenolic antioxidants and initially protects against mitochondrial membrane depolarization. Cancer Lett., 215(1): 43-52.
47. Montague, P.R., C.D. Gancayco, M.J. Winn, R.B. Marchase and M.J. Friedlander, 1994. Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex. Science, 263: 973-977.
48. Ohkuma, S., M. Katsura, J.L. Guo, T. Hasegawa and K. Kuriyama, 1995. Involvement of peroxynitrite in N-methyl-D-aspartate- and sodium nitroprusside-induced release of acetylcholine from mouse cerebral cortical neurons. Mol. Brain Res., 31: 185-193.
49. Zhu, X.Z. and L.G. Luo, 1992. Effect of nitroprusside (nitric oxide) on endogenous dopamine release from rat striatal slices. J. Neurochem., 59: 932-935.
50. Kuriyama, K. and S. Ohkuma, 1995. Role of nitric oxide in central synaptic transmission effects an neurotransmitter release. Jpn. J. Pharmacol., 69: 1-8.
51. Satoh, T., M. Izumi, Y. Inukai, Y. Tsutsumi, N. Nakayama, K. Kosaka, Y. Shimojo, C. Kitajima, K. Itoh, T. Yokoi and T. Shirasawa, 2008. Carnosic acid protects neuronal HT22Cells through activation of the antioxidant-responsive element in free carboxylic acid- and catechol hydroxyl moieties-dependent manners. Neurosci Lett., 434(3): 260-265.
52. Bhale, S.D., Z. Xu, W. Prinyawiwatkul, J.M. King and T.S. Goodber, 2008. Oregano and rosemary extract inhibit oxidation of long – chin n-3- fatty acids in menhaden oil. J. Food. Sci., 72(9): C504-C508.
53. Haraguchi, H., T. Saito, N. Okamura and A. Yagi, 1995. Inhibition of lipid peroxidation and superoxide generation by diterpenoids from Rosmarinus Officinalis. Planta Med., 61(4): 333-336.
54. Park, J.A., S. Kim, S.Y. Lee, C.S. Kim, K. Kim do, S.J. Kim and H.S. Chun, 2008. Beneficial effects of carnosic acid on dieldrin-induced dopamineergic neuronal cell death. Neuroreport, 19(13): 1301-1340