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 Maillard 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
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, anti-tumor, 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 25°C and the dried leaves of plant were ground 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 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 (µg/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 (µg/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 (µg/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 (µg/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 (µg/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 (µg/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 increase 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 decrease 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 demonstrated that the rosemary 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. demonstrated 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 radical 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 influx into neurons in association with opening of voltage-dependent CA channels. The increase in CA influx is assumed to contribute to the initiation of CA 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 moics [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 acid may protect biological membrane from oxidative damage [53]. Park et al reported that carnosic acid 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**


