The effect of aqueous extracts of Fennel (*Foenicul umvulgare* Mill) seeds on some neurotransmitters Content and histological structure changing of cerebellar cortexin the brain of male albino rats

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Abstract: Back ground: Fennel (Foeniculum Vulgare Mill) is amedical and aromatic plant wildly used in Saudi Arabia as traditional medicine for a wide range of diseases. **Objective:** To study the effect of aqueous extract of Foeniculumvulgare Mill. on some neurotransmitters content in all brain region of male albino rats. **Methods:** Forty two male albino rats were divided in to three groups: normal control (contain 6 rats) and treated group (24 rats) receiving orally aqueous extracts of fennel seeds (200 mg/ kg b.w.) for four consecutive weeks and decapitated 6 rats after 1,2,3,4 weeks for physiological study and (12 rats) decapitated 6 rats after 2 and 4 weeks for histological study. **Results**: Results showed that the daily ingestion of aqueous extract of fennel seeds caused significant increase in the total content of neurotransmitters in all the tested regions. This probably due to the presence of active compounds such as Saponin and estragole where worked on the inhibition of release neurotransmitters studied from producing cells resulting in a higher content inside brain cells With the neuroprotective effects of the cerebellar cortex tissue fennel to contain a high amount of antioxidants. **Conclusion:** The study concluded that the fennel extract is likely to be an effective influence as a sedative as well as his protective effects nervous for the maintenance of brain tissue.

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Key words: *Fennel Foeniculum vulgare*, Neurotransmitter, Brain, Rats, cerebellum

1. Introduction:

Fennel (*Foeniculum vulgare* Mill.) is one of the plants commonly used in most countries in the Arab world, especially Saudi Arabia due to his multiple benefits as it is used in many areas, both in food, medical aspects, the pharmaceutical industry, cosmetics, environmental and agricultural aspects (1,2). It's leaves and fruits contain vitamins such as vitamin A, B, C, E (3). Focused medical part of the fennel is seeds and is widely used in many cases, such as dyspepsia, respiratory tract infections, pain and in the treatment of many diseases of the liver (4).

Fennel aromatic and medical oils contains more than 87 species of the major volatile compounds such as estragole, fenchone, Anethole and alpha-phellandrene (5,6,7). Flavonoids are important class of antioxidants in the human diet materials and abundant flavonoids in plants of the family tent, especially fennel (8). Fennel also haveantiseziures potential by injection material Pentylentrazolat dose of (1mg/kg) within the peritoneum of male rats (9). Studies have shown that the use of methanol extract of fennel for eight consecutive days improves memory loss resulting from the materialscopolamine (10)It has an effective impact in alleviating the psychological and physical symptoms associated with the concerned (11).

This study aims to determine the chronic effect of Foeniculumvulgare Mill. aqueous extracts on some neurotransmitter contents norepinephrine (NE), dopamine (DA) and gamma-amino butyric (GABA) in different brain areas and histological changing of cerebellar cortex region in the brain of male albino rats.

2. Materials and Methods:

Animals:

The experimental animals used in this study were male albino rats, Rattusrattus (90 g - 100 g). They were supplied with food and water under standard conditions of light, humidity and temperature (22°C - 25°C).

Preparation of extract:

One kilo gram of dry fennel seeds was obtained from of the local market in Saudi Arabia. It was crushed to obtain the powder and the powder with boiling (5L) of boiling water for 30 minutes and then ran through a mix of pieces of gauze to remove large impurities. Then, it was filtration in Whattman Filter Paper to get rid of all the remaining impurities. Extract were packaged in plastic clean bottles and was given to the test animals at a dose (200 mg / kg b.w) by oral tube so that each 1 ml contains the specific dose depending on the weight of the animal. (12).

Animal Treatment:

Was used in this research 42 young male rats, Rattusrattus weighing approximately (90 - 110 gm). It was be given water and food under the standard conditions of moisture, temperature (22 °C – 25 °C) and light in the Faculty of Science laboratory Al-Faisalyyah Branch of the King Abdul Aziz University in Jeddah, Saudi Arabia.

The experimental animals were randomly distributed into three groups.

The control group containing six rats were treated with saline vehicle and slaughtered at the beginning of the experiment.

The second group containing twenty four rats were administered orally with aqueous extracts of fennel through oral tube for 4 week, and six rats were slaughtered suddenly after 1, 2, 3 and 4 week for physiological study.

The third group containing twelve rats were administered orally with aqueous extracts of fennel through oral tube for 4 week, six rats were slaughtered after 2 and 4 weeks for histological study.

Physiological Studied:

The brain is separated from the rats and dissected quickly on a glass plate cooler according to the method (13) to the following regions: the cerebellum, striatum, cerebral cortex, hypothalamus, brain steam and hippocampus separation of neurotransmitters NE and DA of the brain tissue and assess their content。 According to the method (14-15). separation of neurotransmitter GABA and estimate its content according to the method (16). The fluorescence was measured in Jenway 6200 fluorometer.

Histological Studies (Light microscopic study):

The rats were slaughtered after two and four weeks of treatment for the study of histopathological changes caused by the material used in the study. The head was taken and dissect the skull very carefully to get the cerebellum and then wash with a physiological solution to remove blood and suspended impurities. The parts of cerebellum installed in Boane solution for 24 hours and then wash it and placed in 70% alcohol to remove the yellow color then fixed in formalin and processed for light microscopic study to get sections. Sections were stained with Haematoxylin and Eosin (H&E) (17).

Statistical Analysis:

The data of neurotransmitter contents are presented as mean ±S.E. Statistical analyses between control and treated animals were performed using paired student 't'(18).

3. Results:

Physiological study:

The daily oral administration of aqueous extracts of *Foeniculumvulgare* Mill. (200mg/ kg b.w.) caused a significant increase in NE content starting from the 1st week in the brain area till the end of the experiment duration except hypothalamus. The maximal increase ($p<0.05$) in NE content was found in the cerebral cortex after 3 weeks (104.53%) Table 1, Figure 1.

The data in Table 2, Figure 2 showed that aa considerable increase in DA content from the 1 week in the all brain area after the daily oral administration of Foeniculumvulgare Mill. (200mg/ kg b.w.). The The maximum rise ($p<0.05$) in DA content found after 4 weeks in the cerebral cortex (206.40%). increase in GABA content from the 1st week in the all brain area till the end of the experiment except in hippocampus. The maximum raise in GABA content found after 1 weeks in the cerebellum (46.03%). Table 3, Figure 3.

Table (1): Effect of chronic oral administration of Aqous extract of *Foeniculum Vulgare* Mill. Seeds (200 mg/kg b.w.) on norepinephrine (NE) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	95.382 ± 0.845	511.473 ± 1.803	56.203 ± 0.225	596.997 ± 3.242	390.050 ± 0.831	292.540 ± 1.536
	T	120.167 ± 0.477	565.667 ± 1.256	67.500 ± 0.764	704.333 ± 1.476	437.500 ± 0.847	326.667 ± 0.667
	%	25.99 *	10.60	20.10 *	17.98	12.17 *	11.67
2 weeks	C	95.382 ± 0.845	511.473 ± 1.803	56.203 ± 0.225	596.997 ± 3.242	390.050 ± 0.831	292.540 ± 1.536
	T	120.167 ± 0.477	565.667 ± 1.256	112.667 ± 0.989	704.333 ± 1.476	437.000 ± 0.931	323.333 ± 0.558
	%	25.99 *	10.60	100.46 *	17.98 *	12.04 *	10.53 *
3 weeks	C	98.688 ± 0.274	495.653 ± 1.445	55.493 ± 0.105	604.906 ± 2.337	394.485 ± 0.942	283.178 ± 0.817
	T	163.500 ± 0.428	549.667 ± 1.838	113.500 ± 0.671	702.333 ± 0.882	437.333 ± 0.919	327.333 ± 0.803
	%	65.67 *	10.90 *	104.53 *	16.11 *	10.86 *	15.59 *
4 weeks	C	98.688 ± 0.274	495.653 ± 1.445	55.493 ± 0.105	604.906 ± 2.337	394.485 ± 0.942	283.178 ± 0.817
	T	185.167 ± 0.477	565.000 ± 0.730	110.167 ± 0.654	692.667 ± 0.919	437.667 ± 0.843	337.000 ± 0.730
	%	87.63 *	13.99 *	98.52 *	14.51 *	10.95 *	19.01 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t'* test.
% : Percentage of change from control. * : Significant at $p<0.05$.

Table (2): Effect of chronic oral administration of Aqous extract of *Foeniculum Vulgare* Mill. Seeds (200 mg/kg b.w.) on dopamine (DA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	145.648 ± 0.914	482.312 ± 3.336	61.240 ± 0.214	739.237 ± 4.314	451.541 ± 1.947	244.597 ± 1.448
	T	179.050 ± 0.499	553.667 ± 0.919	68.000 ± 0.365	752.667 ± 0.919	497.000 ± 0.365	270.167 ± 0.477
	%	22.93 *	14.79	11.04 *	1.82	10.07 *	10.45 *
2 weeks	C	145.648 ± 0.914	482.312 ± 3.336	61.240 ± 0.214	739.237 ± 4.314	451.541 ± 1.947	244.597 ± 1.448
	T	167.667 ± 0.955	529.667 ± 0.667	98.000 ± 0.365	763.000 ± 0.966	497.000 ± 0.577	272.333 ± 0.919
	%	15.12 *	9.82	60.03 *	3.21	10.07 *	11.34 *
3 weeks	C	146.755 ± 0.818	473.948 ± 0.856	60.488 ± 0.044	734.223 ± 2.111	451.288 ± 0.633	243.147 ± 0.863
	T	188.333 ± 0.760	551.667 ± 0.558	121.000 ± 0.365	764.333 ± 0.333	505.000 ± 1.826	275.000 ± 0.365
	%	28.33 *	16.40 *	100.04 *	4.10	11.90 *	13.10 *
4 weeks	C	146.755 ± 0.818	473.948 ± 0.856	60.488 ± 0.044	734.223 ± 2.111	451.288 ± 0.633	243.147 ± 0.863
	T	188.333 ± 0.760	548.000 ± 0.365	185.333 ± 0.422	784.000 ± 0.365	521.667 ± 0.558	286.000 ± 0.365
	%	28.33 *	15.62 *	206.40 *	6.78	15.60 *	17.62 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t'* test.
% : Percentage of change from control. * : Significant at p<0.05.

Table (3): Effect of chronic oral administration of Aqous extract of *Foeniculum Vulgare* Mill. seeds (200 mg/kg b.wt.) on gama-aminobutyric acid (GABA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.544 ± 0.759	171.329 ± 0.632	57.041 ± 0.330	432.606 ± 0.326	118.034 ± 0.161	214.766 ± 1.329
	T	281.167 ± 0.477	242.667 ± 0.760	64.000 ± 0.365	477.833 ± 0.654	135.667 ± 0.760	215.000 ± 0.365
	%	46.03 *	41.64 *	12.20 *	10.45 *	14.94 *	0.11
2 weeks	C	192.457 ± 0.799	171.652 ± 0.450	57.247 ± 0.385	432.828 ± 0.319	118.155 ± 0.197	214.787 ± 1.321
	T	212.667 ± 0.919	192.667 ± 0.715	65.500 ± 0.428	476.333 ± 0.760	138.000 ± 0.365	216.000 ± 0.894
	%	10.50 *	12.24 *	14.42 *	10.05 *	16.80 *	0.56
3 weeks	C	193.611 ± 0.781	175.423 ± 1.783	57.849 ± 0.675	437.968 ± 1.007	118.436 ± 0.231	216.865 ± 0.870
	T	217.000 ± 0.365	193.500 ± 0.764	65.167 ± 0.307	491.333 ± 0.558	137.000 ± 0.730	215.500 ± 0.671
	%	12.08 *	10.30 *	12.65 *	12.18 *	15.67 *	-0.63
4 weeks	C	193.379 ± 0.440	171.744 ± 1.615	57.713 ± 0.935	437.849 ± 0.198	118.118 ± 1.398	218.139 ± 2.746
	T	223.833 ± 0.872	190.000 ± 0.365	65.000 ± 0.365	486.833 ± 0.601	134.500 ± 0.847	216.000 ± 0.856
	%	15.75 *	10.63 *	12.63 *	11.19 *	13.87 *	-0.98

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t'* test.
% : Percentage of change from control. * : Significant at p<0.05.

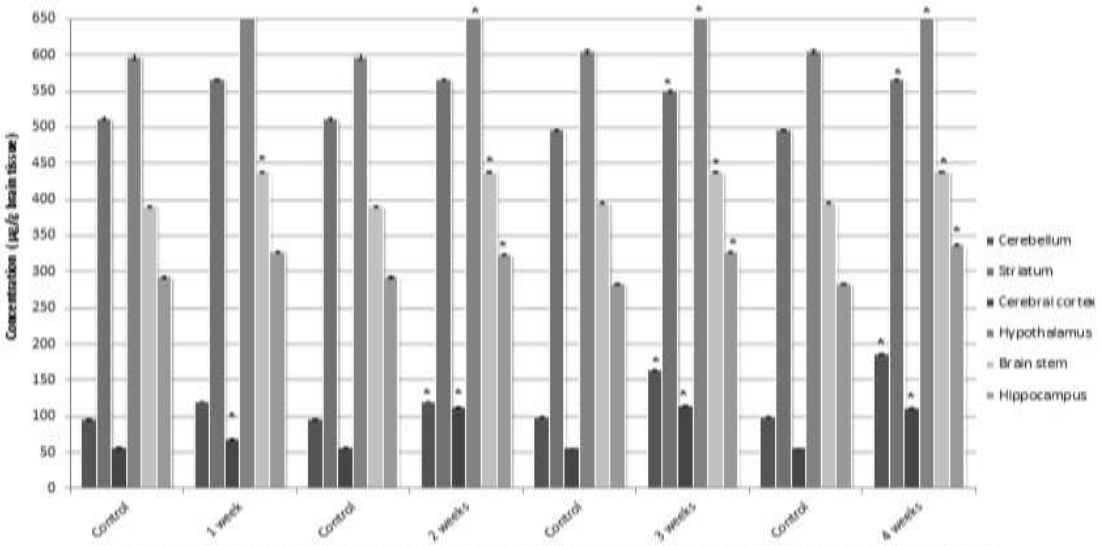

Fig. (1) : Effect of chronic oral administration of Aqous extract of Foeniculum Vulgare Mill. Seeds (200 mg/kg b.w.) on norepinephrine (NE) content in the different brain areas of male albino rat (mean± S.E.).

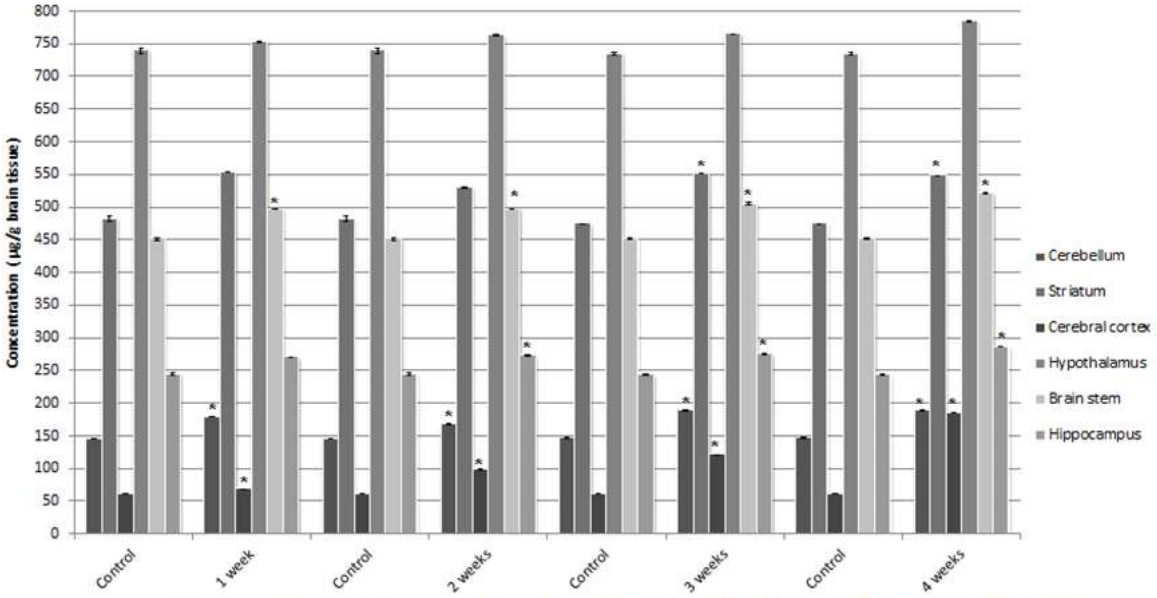

Fig. (2) : Effect of chronic oral administration of Aqous extract of Foeniculum Vulgare Mill. Seeds (200 mg/kg b.w.) on dopamine (DA) content in the different brain areas of male albino rat (mean ± S.E.).

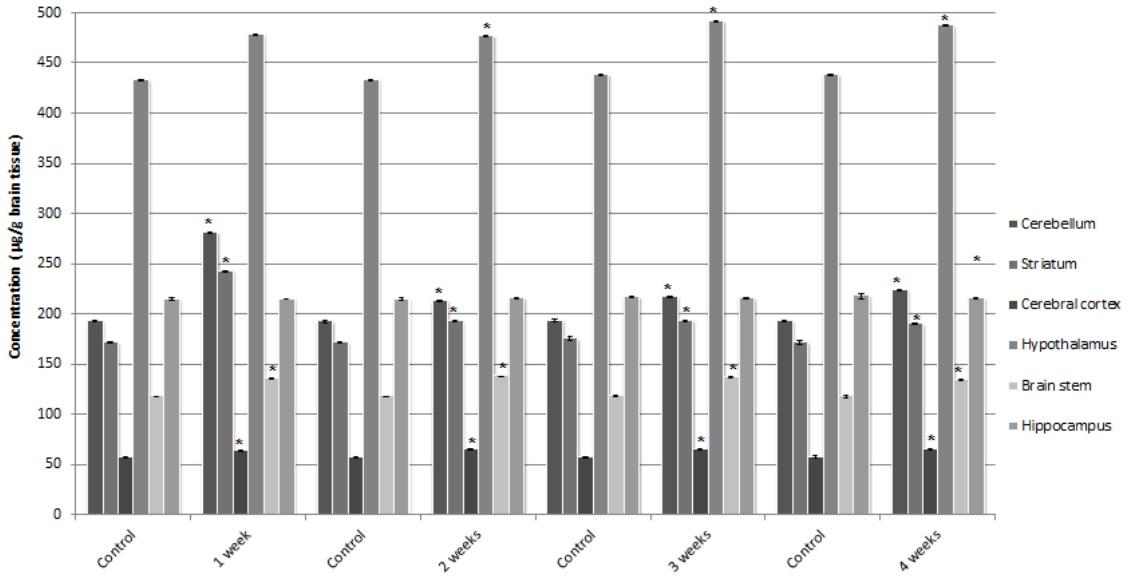

Fig. (3) : Effect of chronic oral addministration of Aqous extract of *Foeniculum Vulgare* Mill. seeds (200 mg/kg b.wt.) on gama-aminobutyric acid (GABA) content in different brain areas of male albino rat (mean ± S.E).

Histological study:

Rats In the control group, cerebellum appeared as irregular folia formed of external gray matter (cerebellar cortex) and internal white matter. The formation of the cerebellar cortex of three layers: outer molecular layer (M), middle Purkinje cell layer (P) and inner granular layer (G) (Fig. 4). Rats treated with aqueous extract of fennel seeds (200 mg / kg bw) for two weeks appeared positive impact on the cerebellar tissue, where there were no histopathological changes clear and cells showed identification to those in the control group form (Figure 5) as the positive effect of aqueous extracts continued after four weeks of treatment and showing no histological changes constantly treatment (Figure 6).

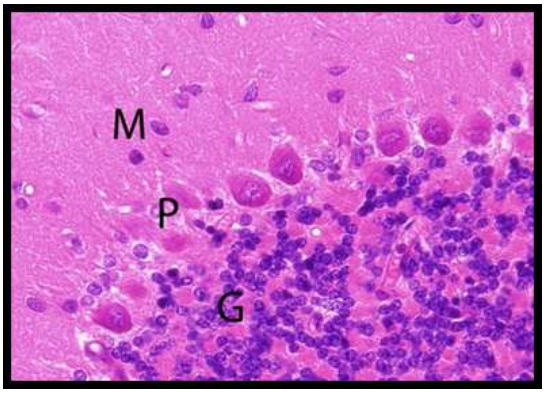

Figure (4):): cerebellar cortex of control group. H&E (X1000)

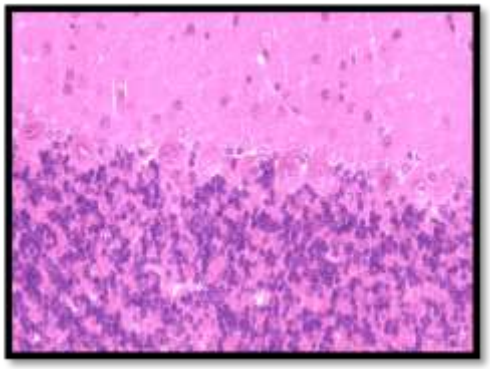

Figure (5): cerebellar cortex in treated animal with fennel (200 mg/kg b.w.) after two weeks (X1000).

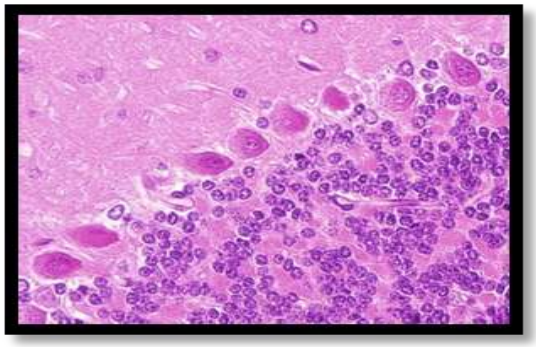

Figure (6): Cerebellar cortex in treated animal with fennel (200 mg/kg b.w.) after four weeks (X1000).

4. Discussion:

The results of the current study showed that chronic treatment aqueous extract of fennel (200 mg / kg bw) caused a significant increase in the total content of norepinephrine, dopamine and gamma-amino butyric acid in the most studied regions of the brain during the treatment period. This is consistent with some previous studies which indicated that the aqueous extract of fennel seeds works as a disincentive for the enzyme monoamine oxidase, which increases the content of DA, NE and serotonin in the brain (19).

The daily ingestion of fennel seeds extract at a dose (300 mg / kg bw) caused a significant increase in monoamines in different regions of the brain content (cerebellum, striatum, cerebral cortex, hypothalamus, brain stem and hippocampus) of adult male rats (20).

Many studies that aim to study effective compounds in fennel plant, which play an important role in plant impact on the different neurophysiological aspects on experimental animals. These studies showed that the compound estragole is one of the components of plants used in traditional medicine, such as fennel and it has many biological and pharmacological properties. Research has shown that it works to neural excitability by direct inhibition in the activity of voltage-gated sodium channels thus inhibition graded for Action potential occurs by affecting the flow of calcium ions (Ca2+) entering and fennel extract effect on calmodulin-dependent Ca2+ through its impact on calcium channels where opening of calcium channels through the electrical alarm to the cell membrane leads to a rush of calcium ions through calcium channels and calcium channels linking to calmudiolin. Calcium associated with calmudiolin caused the transmission of vesicles containing neurotransmitters to the ends of nerve cells. Then linked to cell membranes and released neurotransmitters to the synaptic cleft by Exocytosis and then linked to its receptors in postsynaptic cells. Inhibition of the link between calcium and calmudiolin by inhibiting the enzyme calmodulin-dependent calcium-ATPase leads to release of neurotransmitters from the cells axes ends of pre-synaptic and then content rises within the brain. As a result of the direct effect of Estragole on sodium channels, fennel works as local anesthetic activity and it has no effect on K + channels (21,22).

It is clear from the results of the current histological study that not affected cerebellum region with daily treatment with aqueous extracts of fennel seeds (200 mg / kg bw) for four consecutive weeks may be due to the presence of phenolic compounds, one of the antioxidants that have worked to maintain the nervous tissue(23).

Conclusions

Fennel is likely to be safe for use as a moderator and enhancer of the functions of the central nervous system by increasing the total content of neurotransmitters and also can be work as a protection against stress and stress-related diseases such as memory loss, because it contains high amounts of antioxidants.

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