Regional Brain Amino Acids Imbalances in Reserpine Rat Model of Depression: An Antidepressive Effect of Pomegranate Juice.

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Abstract: Depression is an extremely common pathological complex with psychological, neuroendocrine, and pathological symptoms. The present evaluated the antidepressant effect of pomegranate juice by using the in experimental rat model of reserpinized rat. The occurrence of depression was determined using forced swimming test (FST). Fluoxetine was used as the reference antidepressant drug. In addition the free amino acids (aspartic, glutamic, Glycine. Gamma amino butyric acid, GABA and taurine) content of both brain cortex and midbrain and brainstem were determined. Daily administration of reserpine at a dosage of 1mg/kg for 14 days increased the immobility time in FST without remarkable changes in motor function in the open field test (OFT), indicating the occurrence of depression. Both the individual treatment of fluoxetine and pomegranate juice and their combination significantly reduced the immobility time in FST, indicating their antidepressant activities. In addition, reserpine disturbed the normal levels of both excitatory (glutamic and asparatic) and inhibitory (glycine and GABA) and taurine in both brain cortex and midbrain and brain stem. Both the individual treatment of fluoxetine and pomegranate juice and their combination significantly restored the normal levels of brain free amino acids. The study suggested that reserpine induced depression by altering the normal balance of both brain excitatory and inhibitory amino acids neurotransmission, On the other hand, both the individual treatment of fluoxetine and pomegranate juice and their combination significantly rebalanced the normal brain transmission of excitatory and inhibitory amino acids. The observation that that the combined effect moderately exceeded the individual treatments which might indicate that both fluoxetine shared similar pathways to produced their antidepressive effects, which represent a type of competition. Hence, it is a good opportunity to reduced the fluoxetine dose and compensate by pomegranate as food supplement to attain the full antidepressive effect
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Key words: Depression- reserpine- amino acids- fluoxetine- pomegranate juice.

1. Introduction

Depression is a common, debilitating, life-threatening illness with a significant incidence in the population. Numerous antidepressant compounds are now available, presumably acting via different mechanisms including serotonergic, noradrenergic and/or dopaminergic systems. A large number of experimental and clinical studies indicate that the serotonin (5-HT) system is strongly implicated in the neural regulation of mood and several pieces of evidence have implicated the abnormalities in 5-HT neurotransmission in the pathophysiology of depression (Wong and Licinio, 2001). In accordance, several studies indicate that an enhancement of 5-HT neurotransmission underlies the therapeutic response to various types of antidepressant treatments. Drugs affecting 5-HT neurotransmission, such as those inhibiting 5-HT reuptakes at nerve terminals, or inhibiting its metabolism (monoamine oxidase inhibitors), are effective in depression (Nemeroff and Owens, 2002). Fluoxetine is widely used as an alternative to the older tricyclic antidepressant as it has fewer unpleasant side effects and safer in over dose (Brambilla *et al.*, 2005).

Although a wide variety of antidepressant drugs and antidepressant herbal medicines are available to treat depression, unfortunately the efficacy of these medications is unsatisfactory and multiple side effects are common (Poleszak *et al*., 2004; Chambers *et al*., 2006). Furthermore, these drugs require at least 2–4 weeks of administration before producing clinically meaningful improvement in the symptoms (Skolnick *et al.,* 2009). Therefore, the search for food with antidepressant activity seems to be a mandatory approach to finding an effective antidepressant remedy without side effects.

Pomegranate supplementation decreases oxidative stress and ameliorates impairment in learning and memory performances in diabetic rats indicating its potential as a useful in treatment in neuronal deficit in diabetic patients (Cambay *et al*., 2011). In addition, pomegranate juice has been found

to decrease the amyloid load and improves behavior in a mouse model of Alzheimer's disease (Hartman *et al*., 2006). In addition, Pomegranate induced improvement in sleep disorders in postmenopausal women (Auerbach *et al*., 2012). Moreover, *in vitro* study showed that pomegranate concentration dependently modulates estrogen receptors activity *in-vitro* (Tran *et al*., 2010), which might explain neuroactivity of pomegranate. Consistently, a previous study showed that pomegranate is clinically effective on a depressive state in menopausal syndrome in women (Mori-Okamoto *et al*., 2004). It is worthy to note that fruits, vegetables and cereals contain high levels of polyphenols and fatty acids (Manach *et al*., 2004). Although, literature regarding the antidepressive effects of pomegranate is scarce, its very rich content of polyphenols and fatty acids might underlie this property.

The present study aims to evaluate the effect of individual and combination a natural antidepressant remedy (pomegranate juice) with a reduced dose of conventional antidepressant –fluoxetine-in an attempt to gain a full therapeutic effect and minimal side effect.

2. Materials and Method

Animals

Male adult albino rats 150 ± 10g were used. The animals were brought from laboratory animal breeding of National Organization for Drug Control and Research (NODCAR), Giza, Egypt. Rats were kept under strictly hygienic conditions, fed on a standard basal diet, and allowed free access to drinking water. Before the beginning of the experiment, animals were allowed to adapt to the environment for 2 weeks. The experimental protocols were approved by the NODCAR's Institutional Ethical Guidelines for Animal Care and Usage.

Chemicals

Reserpine and fluoxetine hydrochloride were purchased from Sigma Co. USA.

Reserpine was injected i.p in 5% Dimethyl sulfoxide (DMSO). The pomegranate juice was prepared and administered at dose of 3 ml/kg (Abdel Moneim *et al*., 2011). The tested dose of fluoxetine was equivalent to the daily human dose (El Refaey *et al*., 2011).

Induction of depression: All rats (except 20 rats were left as negative control group) were injected i.p by 1.0 mg/kg/B.wt of reserpine for two weeks, then maintained at 0.1 mg/kg for 14 and 28 days. The negative control group and reserpinized main group was further divided into three groups as follows:

G1, Negative control group (C), fed on basal diet and injected i.p with 5% DMSO (as vehicles).

G2, Positive control group (Res), administrated and injected with vehicles.(n=20).

G3, reserpinized rats were treated with fluoxetine orally (30mg/kg).(n=20).

G4., reserpinized rats were treated with pomegranate juice (3 ml/kg)**(n=20).**

G5, reserpinized rats were treated with a combination of fluoxetine and pomegranate juice, orally (n=20).

At the end of the treatment schedule, animals were subjected to behavioral studies including; Open field test (for studying locomotors activity), forcing swimming test (for studying antidepressant activity), then they were killed by sudden decapitation. The brain was rapidly and carefully excised and then dissected on dry ice glass plate to separate cortex and midbrain and brainstem. Tissues were homogenized in iced 70% methanol, centrifuged and the supernatant was separated. Amino acids were determined in both brain cortex and midbrain and brainstem by using HPLC method according to Heinrikson and Meredith (1984).

Open field Test: Locomotors activity was measured in the open-field test. One hour after the last treatment, all subjects were tested in the open-field apparatus. This test was used to rule out the possibility that a reduction in the immobility time in the FST is due to an increase in the locomotors activity of rats (false positive antidepressant effect). The rats were placed individually in an apparatus consisted of a square arena (It consists of wooden box of squared floor each side is 80 cm and 40 cm hight, with red sides and white floor. The field was divided into 16 equal square 4 x 4 by black lines. Each animal was introduced into the central square of the open field and observed for a period of 3 minutes to record the ambulation frequency which reflects the locomotor activity. It is defined as the number of squares crossed by the animal. The motion path of the rat was continuously traced manually. The open field was cleaned with a water-alcohol (10%) solution before behavioral testing to avoid possible bias due to odors and/or residues left by rat tested earlier (Gould *et al.,* 2009).

Forced Swimming Test: In the present work, rats were placed individually in an acrylic glass tank (50 cm height, 30 cm diameter) filled with water to a depth of 30cm, at 25C°, for 5min. The water is changed between each animal. The water level in the glasses must be high enough to prevent the animal from touching the bottom of the cylinder with his paws or tail, and low enough to avoid an escape through the top opening of the cylinder.

The duration of the immobility was recorded during the last 5 min of the 6 min trial. Immobility time was used as an index of depressive behavior.

Immobility, i.e., when the rat remained floating in the water, making only the necessary movements to keep their heads above water, and the active behaviors include: swimming, i.e., when the animal made active swimming motions; and climbing, i.e., when they made vigorous movements with their forepaws in and out of the water. Following the test, the animals were dried in a warm enclosure (30.0 ±1.0 °C) (Porsolt *et al*., 1977).

Statistical analysis:

Data are expressed as mean ± S.E. All the data were analyzed using one way analysis of variance (ANOVA) followed by determination of least significant difference (LSD) for multiple comparison test. *P*-value < 0.05 was considered significant.

3. Results

Reserpine significantly depleted GABA and taurine and increased the levels of glutamic, aspartic and glycine in brain cortex. The individual treatment fluoxetine and pomegranate and their combination significantly attenuated the effect of reserpine on amino acid levels. The magnitude of the combined treatment was greater (Tables 1, 2, 3, 4 and 5).

Data in tables 6,7, 8, 9 and 10 showed that reserpine significantly depleted GABA and taurine and increased the levels of glutamic, aspartic and glycine in midbrain and brain stem. The individual treatment fluoxetine and pomegranate and their combination significantly attenuated the effect of reserpine on amino acid levels. The magnitude of the combined treatment was greater.

Table 1: Effect of Fluoxetine (Flx, 10mg/kg) and Pomegranate Juice (Pom, 3 ml/kg) alone or in combination on Aspartic Acid level (µg/g tissue) in Brain Cortex of Reserpinized (Res, 1mg/kg) Depressed Rat.

	Aspartic Acid (µg/g tissue)		Aspartic Acid (µg/g tissue)	
Duration Group	**2 weeks mean ± S.E**	**% of Control**	**4 weeks mean ± S.E**	**% of Control**
Control	2.936 ± 0.228	100	2.995 ± 0.233	100
Res	5.455 ± 0.329 *	185.80	6.000 ± 0.362 *	200.33
Res + Flx	3.636 ± 0.111 *, $	123.84	3.309 ± 0.100 $	110.48
Res + Pom	4.272 ± 0.062 *, $	145.50	3.974 ± 0.058 *, $	113.44
Res + Flx+ Pom	3.255 ± 0.080 $	110.87	3.027 ± 0.075 $	101.07

*significant different from control group in the same column
$ significant different from Res group in the same column

Table 2: Effect of Fluoxetine (Flx, 10mg/kg) and Pomegranate Juice (Pom, 3 ml/kg) alone or in combination on Glutamic Acid level (µg/g tissue) in Brain Cortex of Reserpinized (Res, 1mg/kg) Depressed Rat.

	Glutamic Acid (µg/g tissue)		Glutamic Acid (µg/g tissue)	
Duration Group	**2 weeks mean ± S.E**	**% of Control**	**4 weeks mean ± S.E**	**% of Control**
Control	18.447 ± 0.604	100.00	18.815 ± 0.616	100.00
Res	26.457 ± 1.856 *	143.42	28.672 ± 2.267 *	152.39
Res + Flx	19.814 ± 0.558 $	107.41	18.031 ± 0.508 $	95.83
Res + Pom	21.419 ± 0.343 *, $	116.11	19.919 ± 0.319 $	105.87
Res + Flx+ Pom	16.512 ± 0.461 $	89.51	15.356 ± 0.429 *, $	81.62

*significant different from control group in the same column
$ significant different from Res group in the same column

Table 3: Effect of Fluoxetine (Flx, 10mg/kg) and Pomegranate Juice (Pom,3 ml/kg) alone or in combination on Glycine level (µg/g tissue) in Brain Cortex of Reserpinized (Res, 1mg/kg) Depressed Rat.

	Glycine level (µg/g tissue)		Glycine level (µg/g tissue)	
Duration Group	**2 weeks mean ± S.E**	**% of Control**	**4 weeks mean ± S.E**	**% of Control**
Control	1.156 ± 0.046	100.00	1.180 ± 0.047	100.00
Res	1.562 ± 0.041 *	135.12	1.437 ± 0.038 *	121.78
Res + Flx	1.195 ± 0.040 $	103.37	1.302 ± 0.044	110.34
Res + Pom	1.442 ± 0.040 *	124.74	1.543 ± 0.043 *	130.76
Res + Flx+ Pom	1.166 ± 0.105 $	100.87	1.271 ± 0.107	107.71

*significant different from control group in the same column
$ significant different from Res group in the same column

Table 4: Effect of Fluoxetine (Flx, 10mg/kg) and Pomegranate Juice (Pom, 3 ml/kg) alone or in combination on GABA level (µg/g tissue) in Brain Cortex of Reserpinized (Res, 1mg/kg) Depressed Rat.

	GABA (µg/g tissue)		GABA (µg/g tissue)	
Duration Group	2 weeks mean ± S.E	% of Control	4 weeks mean ± S.E	% of Control
Control	1.714 ± 0.036	100.00	1.732 ± 0.115	100.00
Res	1.162 ± 0.035 *	67.79	1.058 ± 0.032 *	61.09
Res + Flx	1.677 ± 0.085 $	97.84	1.827 ± 0.093 $	105.49
Res + Pom	1.567 ± 0.089 $	91.42	1.677 ± 0.095 $	96.82
Res + Flx+ Pom	1.887 ± 0.069 $	110.09	2.019 ± 0.073 *, $	116.57

*significant different from control group in the same column
$ significant different from Res group in the same column

Table 5: Effect of Fluoxetine (Flx, 10mg/kg) and Pomegranate Juice (Pom,3 ml/kg) alone or in combination on Taurine level (µg/g tissue) in Brain Cortex of Reserpinized (Res,1mg/kg) Depressed Rat.

	Taurine level (µg/g tissue)		Taurine level (µg/g tissue)	
Duration Group	2 weeks mean ± S.E	% of Control	4 weeks mean ± S.E	% of Control
Control	27.108 ± 0.593	100.00	27.650 ± 0.604	100.00
Res	17.908 ± 0.367 *	66.06	15.938 ± 0.327 *	57.64
Res + Flx	25.754 ± 0.515 $	95.01	28.072 ± 0.562 $	101.53
Res + Pom	22.339 ± 0.515 *, $	82.41	24.349 ± 0.562 *, $	88.06
Res + Flx+ Pom	31.549 ± 1.291 *, $	116.38	33.442 ± 1.369 *, $	120.95

*significant different from control group in the same column; $ significant different from Res group in the same column

Table 6: Effect of Fluoxetine (Flx, 10mg/kg) and Pomegranate Juice (Pom, 3 ml/kg) alone or in combination on Aspartic Acid level (µg/g tissue) in midbrain and brain stem of Reserpinized (Res, 1mg/kg) Depressed Rat.

	Aspartic Acid (µg/g tissue)		Aspartic Acid (µg/g tissue)	
Duration Group	2 weeks mean ± S.E	% of Control	4 weeks mean ± S.E	% of Control
Control	1.557 ± 0.121	100.00	1.588 ± 0.123	100.00
Res	2.892 ± 0.427 *	185.74	3.181 ± 0.191 *	200.31
Res + Flx	1.928 ± 0.058 *, $	123.83	1.755 ± 0.053 $	110.52
Res + Pom	2.266 ± 0.032 *, $	145.54	2.107 ± 0.030 *, $	132.68
Res + Flx+ Pom	1.726 ± 0.042 $	110.85	1.605 ± 0.039 $	101.07

* Significant different from control group in the same column; $ Significant different from Res group in the same column

Table 7: Effect of Fluoxetine (Flx, 10mg/kg) and Pomegranate Juice (Pom, 3 ml/kg) alone or in combination on Glutamic Acid level (µg/g tissue) in midbrain and brain stem of Reserpinized (Res, 1mg/kg) Depressed Rat.

	Glutamic Acid (µg/g tissue)		Glutamic Acid (µg/g tissue)	
Duration Group	2 weeks mean ± S.E	% of Control	4 weeks mean ± S.E	% of Control
Control	13.880 ± 0.454	100.00	14.158 ± 0.464	100.00
Res	19.905 ± 1.397 *	143.41	21.574 ± 1.706 *	152.38
Res + Flx	14.909 ± 0.420 $	107.41	13.567 ± 0.382 $	95.83
Res + Pom	16.117 ± 0.258 *, $	116.12	14.989 ± 0.240 $	105.87
Res + Flx+ Pom	12.424 ± 0.347 $	89.51	11.555 ± 0.323 *, $	81.61

*significant different from control group in the same column
$ significant different from Res group in the same column

Table 8: Effect of Fluoxetine (Flx, 10mg/kg) and Pomegranate Juice (Pom, 3 ml/kg) alone or in combination on Glycine level (µg/g tissue) in midbrain and brain stem of Reserpinized (Res,1mg/kg) Depressed Rat.

	Glycine (µg/g tissue)		Glycine (µg/g tissue)	
Duration Group	2 weeks mean ± S.E	% of Control	4 weeks mean ± S.E	% of Control
Control	1.250 ± 0.050	100.00	1.275 ± 0.051	100.00
Res	1.688 ± 0.045 *	135.04	1.553 ± 0.041 *	121.80
Res + Flx	1.291 ± 0.043 $	103.28	1.408 ± 0.048	110.43
Res + Pom	1.559 ± 0.043 *	124.72	1.668 ± 0.046 *	130.82
Res + Flx+ Pom	1.187 ± 0.155 $	94.96	1.270 ± 0.166 $	99.61

*significant different from control group in the same column
$ significant different from Res group in the same column

Table 9: Effect of Fluoxetine (Flx, 10mg/kg) and Pomegranate Juice (Pom, 3 ml/kg) alone or in combination on GABA level (µg/g tissue) in midbrain and brain stem of Reserpinized (Res, 1mg/kg) Depressed Rat.

Duration Group	2 weeks mean ± S.E	% of Control	4 weeks mean ± S.E	% of Control
Control	3.272 ± 0.069	100.00	3.305 ± 0.219	100.00
Res	2.218 ± 0.067 *	67.79	2.019 ± 0.061 *	61.09
Res + Flx	3.200 ± 0.163 $	97.80	3.488 ± 0.177 $	105.54
Res + Pom	2.990 ± 0.170 $	91.38	3.200 ± 0.182 $	96.82
Res + Flx+ Pom	3.602 ± 0.130 $	110.09	3.854 ± 0.140 *, $	116.61

*significant different from control group in the same column
$ significant different from Res group in the same column

Table 10: Effect of Fluoxetine (Flx, 10mg/kg) and Pomegranate Juice (Pom, 3 ml/kg) alone or in combination on Taurine level (µg/g tissue) in midbrain and brain stem of Reserpinized (Res, 1mg/kg) Depressed Rat.

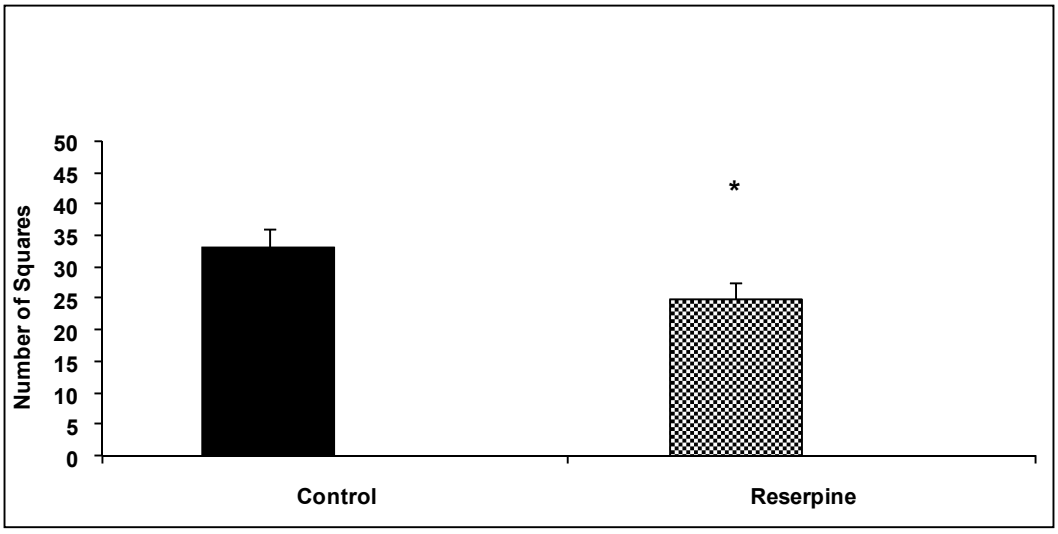
	Taurine (µg/g tissue)		Taurine (µg/g tissue)	
Duration Group	2 weeks mean ± S.E	% of Control	4 weeks mean ± S.E	% of Control
Control	26.933 ± 0.589	100.00	27.472 ± 0.600	100.00
Res	17.793 ± 0.365 *	66.06	15.836 ± 0.325 *	57.64
Res + Flx	25.588 ± 0.512 $	95.00	27.891 ± 0.559 $	101.53
Res + Pom	22.195 ± 0.512 *, $	82.41	24.192 ± 0.558 *, $	88.06
Res + Flx+ Pom	31.346 ± 1.283 *, $	116.39	33.227 ± 1.360 *, $	120.95

*significant different from control group in the same column
$ significant different from Res group in the same column

Table 11: Effect of Fluoxetine (Flx,10mg/kg), Pomegranate Juice (Pom,3 ml/kg) alone or in combination on locomotor activity of Reserpinized (1mg/kg) treated Rat.

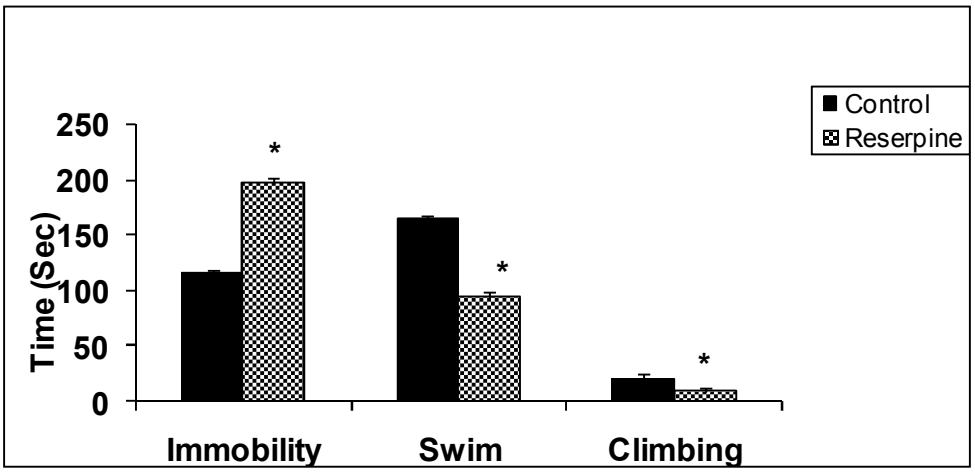
	locomotor activity after		locomotor activity after	
Duration Group	2 week mean ± S.E	% of Control	4 weeks mean ± S.E	% of Control
Control	40.833 ± 3.167	100.00	44.500 ± 5.130	100.00
Res	25.667 ± 3.870 *	62.86	23.000 ± 4.336 *	51.69
Res + Flx	28.333 ± 3.612 *	69.40	26.667 ± 3.303 *	59.92
Res + Pom	28.000 ± 4.033 *	68.57	29.167 ± 6.145 *	65.54
Res + Flx+ Pom	23.500 ± 1.668 *	57.55	30.333 ± 3.252 *	68.16

*significant different from control group in the same column
$ significant different from Res group in the same column



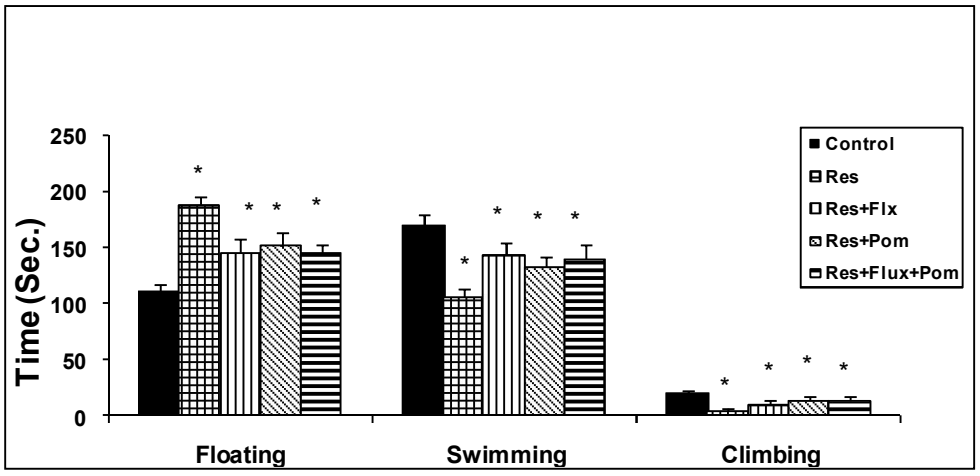
*significant different from control group

Figure 1: Effect of Reserpine (1mg/kg for 14 days) on Locomotor Activity in Open Field Test in Adult Rat.



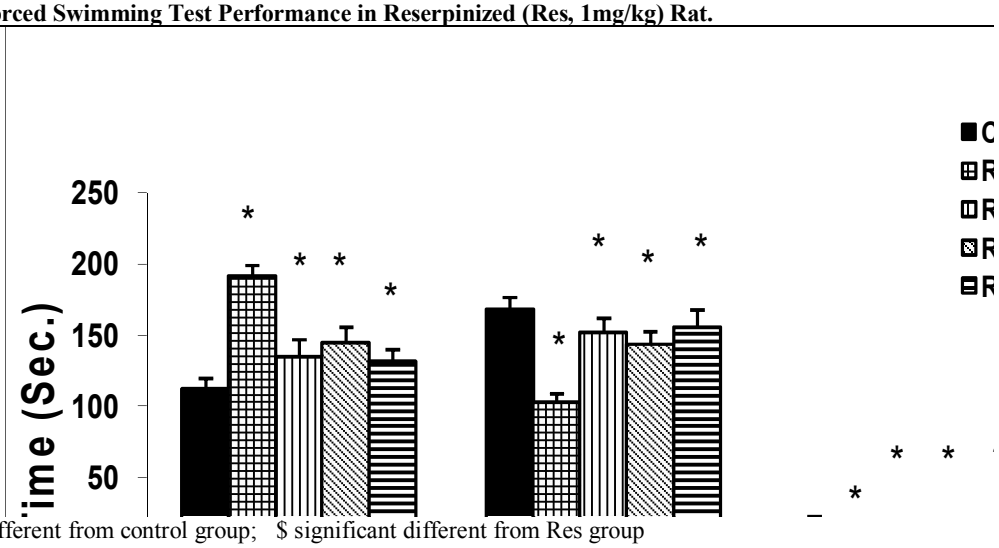
*significant different from control group

Figure 2: Effect of Reserpine (1mg/kg for 14 days) on Adult Rat Performane in Forced Swimming Test.



*significant different from control group; $ significant different from Res group

Figure 3: Effect of Fluoxetine (FLX, 10 mg/kg) and Pomegranate Juice (Pom, 3ml/kg) alone or in Combination for two Weeks on Forced Swimming Test Performance in Reserpinized (Res, 1mg/kg) Rat.



significant different from control group; $ significant different from Res group

Figure 4: Effect of Fluoxetine (FLX, 10 mg/kg) and Pomegranate Juice (Pom, 3ml/kg) alone or in Combination for foor Weeks on Forced Swimmin

4. Discussion

The present study used reserpine model (1mg/kg, p.o. for 14 days) to induce experimental model for depression. The occurrence of depression was justified by using enforced swimming test and the locomotor activity as a measure of the muscle activity determined using open field test. Thereafter, the depressed animals maintained on reduced reserpine dose (0.1mg/kg/ day) and treated with either fluoxetine, pomegranate juice alone or in combination for 14 and 28 days. Reserpinzed rats (1mg/kg, p.o. for 14 days) showed significant decrease in the time spent in climbing and swimming and increased floating time in comparison with control reflecting the reliability of reserpine to induce depression. In addition, the reduced reserpine dose (0.1 mg/kg/day/kg, p.o. for 14 and 28 days) maintained reserpine's effect regarding forced swimming test (FST) parameters. The behavioral performance of reserpinized rat as reduced time spent in climbing and swimming, and increased floating time, compared with control might indicate a state of despair reflecting the occurrence of depression. The observation that reserpine significantly decreased the animals locomotor activity in open field test might indicate that the increased immobility time is attributed to the occurrence of depression from one side and the likely to the affected muscle activity from the other side. In accordance to the present results, several studies used reserpine to induce depression in rodents (Lee *et al.,* 2012, Antkiewicz-Michaluk *et al.,* 2014).

In the present study, reserpine treatment to rats significantly increased the levels of asparatic acid, glutamic acid and glycine and decreased the levels of both GABA and taurine in both brain cortex and midbrain and brainstem after 2 and four weeks in reserpinized depressed rats. This might indicate that the imbalance between the excitatory and inhibitory neurotransmitter amino acids leads to depression. In addition, the decreased level of taurine might suggest the existence of a correlation between the decreased level of taurine and depression. In keeping with the present study, Wu *et al.* (2012) indicated that depressed rats exhibited increased levels of glutamic and asparatic and decreased levels of GABA and taurine in the brain hippocampal region. Moreover, Quines et al. (2014) found that monosodium glutamate (MSG) exposure increased the immobility time in the forced swimming test (FST), and increased the [(3)H]5-HT uptake in the cerebral cortices of rats and induced a deregulation of HPA axis function (by increasing serum ACTH and corticosterone levels. These effects were suggested to represent a dysfunction in the serotonergic system. Furthermore, Taurine is one of the most abundant amino acids in the central nervous system, and it has various important functions as a neuromodulator and antioxidant. Toyoda and Iio (2013) indicated that taurine (22.5 mmol/kg and 45 mmol/kg) supplementation had an antidepressant-like effect and an ability to change that depression-related signaling cascades in the rat hippocampus.

The present study showed that fluoxetine administration to reserpinized rats for 14 and 28 days minimized the depressive effect of reserpine. Fluoxetine effects were increased swimming and climbing time and decreased immobility time in the forced swimming test, in comparison to reserpinized depressed rats. These effects were time dependent and are attributed to the antidepressive effect of fluoxetine. In accordance, several studies indicated the efficiency of fluoxetine in treating major depression (Harkin *et al.,* 2003, Küçükibrahimoğlu *et al.,* 2009, Dhingra *et al.,* 2012; Moretti *et al.,* 2012; Bhatt *et al.,* 2014).

In the present study, fluoxetine treatment for 14 and 28 days remarkably reversed reserpine's effect on amino acids levels. Fluoxetine treatment increased the levels of GABA and Taurine and decreased the levels of glutamic and asparatic acids and glycine. It seems that fluoxetine's reversing effect for reserpine's upon amino acids levels might play a role in the antidepressive effect of fluoxetine. Also, it is plausible the existence of a correlation between the antidepressive effect of fluoxetine and its effects on monoamines and amino acids levels.

In accordance, fluoxetine administration to depressed rats (10 mg/ kg) for 21 days produced remarkable decreases in the levels of Glu and Asp and increased the GABA and Tau in rat hippocampus (Wu *et al.,* 2012). Consistently, oral fluoxetine administration (5 mg/kg) for 21 days also elevated the CSF GABA levels by approximately 2-fold ($P < 0.05$) in adult rats (Gören *et al.,* 2007). These neurochemical findings show that fluoxetine, a selective serotonin re-uptake inhibitor affects brain GABA levels, which may be involved in the beneficial and/or adverse effects of the drug. In addition, taurine is one of the most abundant amino acids in the central nervous system (Iio *et al.,* 2012, Toyoda and Iio, 2013) and is thought to play an essential role in depressive disorders. In accordance, taurine supplementation has an antidepressant-like effect and an ability to change depression-related signaling cascades in the hippocampus by increasing the phosphorylation levels of extracellular signal-regulated kinase1/2 (ERK1/2), protein kinase B (Akt), glycogen synthase kinase3 beta (GSK3β) and cAMP response element-binding protein (CREB). Moreover, taurine feeding increased Phospho-calcium/calmodulin-dependent protein kinase II (CaMKII) in rat hippocampus (Toyoda and Iio, 2013). Moreover, the antidepressive activity of taurine might be- partly- due to the fact that taurine

acts as an agonist at GABAA receptors (Caletti *et al.,* 2012).

The present data showed that pomegranate juice remarkably alleviate reserpine induced depression in rats in a time dependent manner. The antidepressive effect of pomegranate juice was to a great extent comparable to fluoxetine's. It is likely that the antidepressive effect of pomegranate is related o its effect on the monoamines system or amino acids systems in the brain. In a good keeping with the present interpretation, Cambay *et al*. (2011) indicated that pomegranate fruit supplementation decreases oxidative stress and ameliorates impairment in learning and memory performances in diabetic rats and suggested that pomegranate supplementation may be clinically useful in treating neuronal deficit in diabetic patients. A previous study showed that pomegranate is clinically effective on a depressive state in menopausal syndrome in women (Mori-Okamoto *et al*., 2004). Moreover, Kumar *et al*. (2008) showed that pomegranate extract (250 and 500 mg/kg) was able to induce a significant decrease in the immobility time in tail suspension test, similar to imipramine, a recognized antidepressant drug.

Furthermore, in the present work, pomegranate noticeably inversed the effect of reserpine on amino acid levels, in terms, it increased the levels of GABA and taurine and decreased the levels of asparatic, glutamic acids and glycine. This effect might correlate well with the antidepressive effect of pomegranate. This antidepressive effect was comparable to that of fluoxetine. In this respect, it is essential to emphasize that active neurotransmission of both taurine and GABA and normal glutamergic neurotransmission are essential for normal mood and emotionality. On the other hand, inhibited GABA and taurine systems and hyperactive asparatic, glutamic acids and glycine systems produce a state of depression.

It is worthy to note that pomegranate is a rich source polyphenols, which are believed to be responsible for the estrogenic activity. In addition, pomegranate contains steroidal estrogen, estrone and non- steroidal phytoestrogens (Jurenka., 2008). Accordingly, the present study suggest that the antidepressant effect of pomegranate might be attributed- at least partly- to the steroidal estrogen, estrone and non- steroidal phytoestrogens in the pomegranate. The previous revelation shows that estrogens (ER) play essential role, probably due to ER direct and/or indirect effects in the brain, where these hormones act through both genomic (i.e. interaction as transcription factors with nuclear receptors ER-alpha and ER-beta) and non-genomic (i.e. binding with cell-membrane receptors) mechanisms.

The combined treatment remarkably abolished the depressive effect of reserpine in the forced swimming test parameters and normalized the levels of both monoamines and amino acids. It worthy to note that the combined effect moderately exceeded the individual treatments which might indicate that both fluoxetine shared similar pathways to produced their antidepressive effects, which represent a type of competition. Hence, it is a good opportunity to reduced the fluoxetine dose and compensated by pomegranate as food supplement to attain the full antidepressive effect.

Actually, there are two benefits, the first is to reduce the drug dose and consequently its side effect; the second it to get benefited from the beneficial ingredients existed in pomegranate juice. Moreover, pomegranate is a potent antioxidant equal to or better than green tea. In addition, anticarcinogenic and anti-inflammatory properties suggest its possible use as a therapy or adjunct for prevention and treatment of several types of cancer and cardiovascular disease

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