Oral supplementation of *Thuja oreintalis* leaf extract moderately affects the behavior in albino mice in a gender specific manner

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Abstract: Present study was designed to demonstrate the effect ethanolic leaf extract of *Thuja orientalis* on the learning and memory formation in albino mice. Four week old albino mice were either orally supplemented with 50mg/ml solvent/Kg body weight of *Thuja orientalis* or with equal volume of saline solution (0.9% NaCl) for seven days. A battery of neurological tests (Elevated plus maze, Rota Rod, light and dark box, open field and novel object recognition test) were applied at the end of dose supplementation. *Thuja orientalis* leaf extract treated female mice spent significantly les time on rotating rod ($P = 0.02$) than male treated with saline solution [indicating poor neuromuscular coordination]. While all other neurological test performances varied non significantly ($P > 0.05$) when compared between *Thuja orientalis* treated albino mice (of both genders) with their respective control groups indicating a moderate effect of 50mg/ml solvent/Kg body weight of *Thuja orientalis* on the behavior of young albino mice.

Keywords: *Thuja orientalis*, Albino mouse, rota rod, open field, elevated plus maze, light dark box, novel object test.

INTRODUCTION

A wide variety of medicinal plants are being used in therapeutical, pharmaceutical and medicinal fields (Butlet, 2004). According to a recent report from the world health organization (WHO), 80 % of the world inhabitants relying mainly on medicinal plants for their basic health care (Owolabi *et al*., 2007). Medicinal plants contains a variety of chemical substances (e.g. alkaloids, flavanoids, tannis and phenolic compounds) that have definite physiological effects in the human body (Edeoga *et al*., 2005; Abbasi *et al*., 2011).

In Pakistan, it has been reported that 600 to 700 plant species are used for medicinal purpose. *Thuja orientalis* is one of the commonly found medicinal plants in Pakistan and used in traditional medicines as well in homeopathy (Shinwari, 2010). In traditional practices *Thuja orientalis*is used for treatment of enuresis, bronchial catarrh, psoriasis, cystitis, amenorrhea, uterine carcinomas and rheumatism (Wendakoon *et al*., 2012). *Thuja orientalis* is used internally in the treatment of hemorrhages, coughs, bronchitis, asthma, excessive menstruation, skin infections, bacterial dysentery, mumps, arthritic and premature blandness (Srivasta *et al*., 2012). The leaves of *Thuja orientalis* are antipyretic, antifungal, antimicrobial, astringent, diuretic, emmenagogue, emollient, expectorant and stomachic (Saharan *et al*., 2012; Mamidala and Gujjeti, 2013).

Despite of its much medicinal importance, a little is known about the effect of *Thuja orientalis* leaf extract on behavior, learning process and neuromuscular coordination. Aim of this study was to demonstrate the effects of *Thuja orientalis* leaf extract supplementation (@ 50 mg/ml solvent/ Kg body weight) on selected aspect of albino mice behavior in a gender specific manner.

MATERIAL AND METHODS

Subjects

Four-week-old Albino mice [N=20 for male and female] were used during present study. Mice were maintained in wood chips filled cages at the core Animal facility of Bahauddin Zakariya University, Multan, Pakistan. Standard rodent diet and water ad libitum was provided to mice and they were housed individually in cages. Room temperature was maintained at 22±1°C.

Experimental design

On 20th day of life, mice were separated from their parents and fed on normal mouse diet until 4th week of life when each mouse was weighted and orally supplemented either with 50mg/ml solvent/kg body weight of *Thuja orientalis* leaf extract or saline solution (Otsuka, Pakistan) for 7 days prior to behavioral testing.

Thuja oreintalis extract preparation

The leaves of *Thuja oreintalis* plant used in this study were collected from local fields of Multan and subjected to shade drying and rendered free of adulterated material through manual picking. Leaves were taxonomically identified by a taxonomist from department of Botany, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan.

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For the preparation of extract, leaves were weighed and dried in shade for seven days. Dried leaves were powdered with the help of Blender. In amber colored glass bottles, the leaves of *Thuja oreintalis,* 50gin powdered form, were soaked in 70% aqueous-methanol (v/v) and 30% distilled water for 15 days. Occasional shaking was done at least 3-4 times in a day and bottle lid was opened for a moment after every shake to remove the gas. Double-layered muslin cloth was used to pass the soaked material through it. The resultant fluid was filtered through filter paper in order to remove vegetative debris (Williamson *et al*., 1998). The residual material was re-soaked for next three days and the same procedure was repeated. Under reduced pressure (-760mm Hg) at 37^{o}C, the filtrates were evaporated on a rotator evaporator (BUCHI, Switzerland) to prepare a thick semi solid pastes. The thick pastes were put in respective petri-plates for further evaporation. Concentrated extracts were stored at 4^{0}C until tested and utilized.

Rota rod

The Rota rod is a test balance and neuro-muscular coordination in rodents and the apparatus comprises of locally manufactured rotating drum with maximum acceleration of 40 rpm. There were three training trials for each animal followed by three more consecutive test trials and average time spend on the drum, during test trials, was used for analysis following Sunyer *et al* (2007).

Open field (OF)

Open field test is used to study the locomotory and exploratory behavior in rodents. Video monitoring system consisting of a video camcorder (Addlink, Barcelona) coupled to computational tracking system Any Maze (Stoeling CO, IL, USA) in an area (40cmx40cm long with 70cm high walls) was used to observe each mouse for 10min. Each mouse was placed in the middle of the chamber during the trial. Standard parameters for locomotors activity and exploratory behavior (mean speed, maximum speed, total distance covered, time immobile, time mobile, mobile episodes, immobile episodes, rotations, clockwise rotations, anti clockwise rotations) were recorded following Iqbal *et al* (2014).

Light dark box

The light/dark box was made up of plywood, had dimensions 45x27x27cm and consisted of two chambers connected with each other through a small opening (7.5x7.5cm) located in the center of the dividing wall. The floor was covered with Plexiglas and was sub divided into 9x9cm squares. The dark chamber was smaller (18x27cm) light chamber (27 x 27cm) that was painted white. Mouse was placed in the white chamber and the amount of time spent in each chamber, rearing, stretch attend frequency, defecation, urination and the number of transitions between chambers were measured over a 5 minute test following Zahra *et al.* (2014).

Elevated plus maze

Elevated plus maze had a black Plexiglas floor with a 5 x 5cm central square platform from which radiated two 45 x 5cm open arms with 0.25cm high edges and two 45 x 5 cm closed arms with 40cm high walls made of clear Plexiglas. Mouse was allowed to explore for 5 minutes followed by their placement in the central square of the Plus-Maze facing an open arm. A video camcorder located above the center of the maze also recorded behavior. Head-dips, urination and defection were scored by the experimenter, whereas measures of arm entries and the time in each arm was recorded with the Limelight video camera (Addlink, Barcelona) based tracking system (Anymaze, USA).

Novel object test

Novel object recognition is a memory task that is independent of spatial cues. Mice were trained to recognize certain objects in an area (40 cm x 40 cm long with 70 cm high walls) for two trials each consisting of 5 minutes with an interval of 20 minutes between the trials. The individual mouse was placed in the middle of the chamber for each trial. In the first trial, line cross, stretch attend frequency, approach to object A, approach to object B, time spent near object A and time spent near object B were calculated. During the second testing phase, one of the old objects was replaced with a novel object and line cross, stretch attend frequency, approach to old object, approach to novel object, time spent near old object and time spent near novel object were observed.

STATISTICAL ANALYSIS

Minitab, Statistical package, (Version 16, USA) was used for data analysis. All the data were presented as mean ± standard deviation (SD). To compare various parameters of Rota rod, Open field, Elevated plus maze, Light and dark box and Novel object test, 2-sample t-test was calculated for *Thuja oreintalis* and saline treated male albino mice.

RESULTS

Rota rod

Results of the rota rod test indicated that *Thuja orientalis* (50mg/ml solvent/ml body weight) treated female albino mice spent significantly (P=0.02) reduced time on rotating rod than their control group. Male mice supplemented with leaf extract had slightly improved rota rod performance than control but this difference in performance between the two treatments did not reached statistical significance (P=0.7) (fig. 3.1).

Open field

Open field test results indicated a non-significant effect of *Thuja orientalis* leaf extract supplementation on all the studied parameters, in both genders, when compared with their respective controls (Supplementary table 1). *Thuja orientalis* treated female albino mice had more urination

Supplementary Table 1: Comparison of various studied parameters between *Thuja orientalis* (50 mg/ml solvent/Kg body weight) and saline treated adult male and female albino mice during open field test (OF). N=10 for all experimental treatments. All values are expressed as mean ± standard deviation. P-value indicates the results of the 2-sample t-test.

Parameters	Saline treated male	*T. orientalis* treated male	P-value	Saline treated female	*T. orientalis* treated female	P-value
Distance (m)	19.43±5.75	19.59±8.17	0.97	17.08±6.41	19.2±10.7	0.69
Mean Speed (m/s)	0.03±0.00	0.03±0.01	0.98	0.03±0.01	0.03±0.02	0.69
Time mobile (sec)	481.7±68.0	463.8±99.6	0.72	430.3±91.2	418±129	0.86
Time immobile (sec)	118.3±68.0	136.2±99.6	0.72	169.7±91.2	182±129	0.86
Mobile episodes	26.8±13.0	30.2±18.0	0.72	37.2±13.3	34.8±18.1	0.8
Rotation	22.67±5.54	23.0±9.90	0.94	21.5±8.83	22.7±10.9	0.84
Clockwise rotation	12.33±3.44	12.17±5.42	0.95	11.0±4.2	12.5±6.98	0.66
Anti clockwise rotation	10.33±2.80	10.83±5.78	0.85	10.5±5.01	10.17±5.42	0.91
Urination	0.33±0.816	0.33±0.816	1.0	0.5±0.55	1.0±0.9	0.28
Defecation	3.83±3.06	7.00±3.22	0.12	2.4±0.5	5.0±3.58	0.13

P > 0.05 = Non significant

Supplementary Table 2: Comparison of various studied parameters between *Thuja orientalis* (50 mg/ml solvent/Kg body weight) and saline treated adult male and female albino mice during Light dark box. N = 10 for all treatments. All values are expressed as mean ± standard deviation. P-value indicates the result of 2 sample t-test.

Parameters	Saline treated male	*T. orientalis* treated male	P-value	Saline treated female	*T. orientalis* treated female	P-value
Transition frequency	9.17±3.19	14.0±8.74	0.25	18.2±10.2	12.67±4.18	0.27
Rearing frequency	2.0±1.55	1.0±1.26	0.25	2.17±1.94	3±2.28	0.51
Stretch attend frequency	3.0±2.61	6.0±3.58	0.13	5.5±2.43	5±4.34	0.81
Time in dark (sec)	172.3±82.4	203.2±57.7	0.47	168.2±43.7	189.8±28.4	0.34
Time in light (sec)	127.7±82.4	96.8±57.7	0.47	131.8±43.7	110.2±28.4	0.34
Urination	0	0	0	0	0	0
Defecation	4.33±2.16	2.83±2.32	0.28	1.67±1.21	3.33±1.97	0.12

P >0.05 = Non significant

Table 1: Comparison of various studied parameters between *Thuja orientalis* (50 mg/ml solvent/Kg body weight) and saline treated adult male and female albino mice during elevated plus maze. N = 10 for all treatments. All values are expressed as mean ± standard deviation. P-value indicates the value of 2-sample t-test.

Parameters	Saline treated male	*T. orientalis* treated male	P-value	Saline treated female	*T. orientalis* treated female	P-value
Distance (m)	9.96±3.39	12.21±6.63	0.5	15.89±4.85	22.9±29.8	0.6
Mean Speed (m/s)	0.03±0.01	0.04±0.02	0.5	0.05±0.02	0.07±0.1	0.6
Time mobile (sec)	200.0±41.7	190.7±68.8	0.8	181.1±54.8	221±31.9	0.2
Time immobile (sec)	100.0±41.7	109.3±68.8	0.8	118.9±54.8	79±31.9	0.2
Mobile episodes	16.67±4.89	16.33±2.73	0.9	14.83±3.87	16.17±4.79	0.6
Immobile episodes	16.17±5.23	15.50±2.88	0.8	14.17±4.02	15.83±4.79	0.5
Rotation	9.17±2.32	6.00±2.19	0.03*	6.67±4.27	9.83±4.71	0.3
Clockwise rotation	5.83±1.47	3.67±2.58	0.1	4.33±3.08	5.50±2.88	0.5
Anticlockwise Rotation	3.33±1.51	2.33±1.37	0.3	2.33±1.37	4.33±2.07	0.08
Urination	0	0	0	0	0	0
Defecation	1.50±1.38	2.67±1.21	0.15	3.67±2.58	5.67±1.51	0.14
Head dipping	10.17±7.14	12.5±15.9	0.75	10.17±6.85	18.83±7.76	0.07

P > 0.05 = Non Significant; P ≤ 0.05 = Least Significant (*)

Table 2A: Comparison of various studied parameters between *Thuja orientalis* (50 mg/ml solvent/Kg body weight) and saline treated adult male and female albino mice during trail 1 of novel object test. N = 10 for all treatments. All values are expressed as mean ± standard deviation. P- value indicates the result of 2 sample t-test.

Parameters	Saline treated male	*T. orientalis* treated male	P-value	Saline treated female	*T. orientalis* treated female	P-value
Line cross	11.67±5.47	16.50±8.17	0.26	22.2±11	18±6.23	0.45
Stretch attend reflex	3.50±2.59	5.17±2.48	0.28	4.5±1.52	4.33±2.42	0.89
Approaches object A	5.0±3.16	9.17±4.17	0.08	10.83±5.34	10.17±2.71	0.79
Approaches object B	4.17±2.79	11.50±5.36	0.02*	27±31.3	9±3.35	0.22
Time object A (sec)	17.1±18.2	38.0±22.8	0.11	37.2±20	47.2±17	0.37
Time object B (sec)	27.7±26.3	54.1±28.2	0.13	53.2±20.1	48.7±16.5	0.68

P > 0.05 = Non significant; P ≤ 0.05 = Least significant (*)

Table 2B: Comparison of various studied parameters between *Thuja orientalis* (50 mg/ml solvent/Kg body weight) and saline treated adult male and female albino mice during trail 2 of novel object test. N = 10 for all treatments. All values are expressed as mean ± standard deviation. P- value indicates the value of 2-sample t-test.

Parameters	Saline treated male	*T. orientalis* treated male	P-value	Saline treated female	*T. orientalis* treated female	P-value
Line cross	10.50±5.47	9.17±5.19	0.68	13.7±10.2	8±7.13	0.3
Stretch attend reflex	4.0±2.37	5.0±3.52	0.58	5.5±2.88	4.5±3.51	0.6
Approaches Old object A	4.33±2.94	5.0±3.90	0.75	5.67±3.98	4.6±4.27	0.68
Approaches Novel object	5.17±2.14	3.50±1.76	0.17	5.33±3.72	4.5±3.08	0.68
Time Old object (sec)	36.5±34.9	36.0±33.1	0.98	64.7±66.7	35±30	0.36
Time Novel object (sec)	32.0±15.3	41.9±23.8	0.42	54.3±41.7	50.9±70	0.92

P > 0.05 = Non significant

and defecation as compared to their control group indicating anxiety. Over all, male mice treated with leaf extract showed better exploratory behavior but all the studied parameters remained statistically non significant upon comparison with control group (Supplementary table 1).

Light dark box

Light dark box test results revealed that *Thuja orientalis* treated male albino mice had more transition frequency (P = 0.25) but spent most of their time in dark (P = 0.47) as compared to saline treated controls, while saline treated female mice had higher transition frequencies, spent less time in dark and more in light as compared to *Thuja orientalis* treated female albino mice but statistically all the studied parameters varied non significantly in both genders when compared between the control and plant extract treated animals (Supplementary table 2).

Elevated plus maze

Elevated plus maze test data analysis revealed that *Thuja oreintalis* treated male albino mice covered more distance (P=0.5) and had more head dips (P=0.8) indicating better exploration but saline treated male remained mobile for longer time (P=0.8) with more mobile episodes (P=0.89) and rotations (P=0.03). While performance of *Thuja orientalis* treated female albino mice was better as they covered more distance (P=0.6), had more mobile episodes (P=0.6), more head dips (P=0.07) and more rotations (P=0.3) than saline treated female albino mice but most of the studied parameters did not reached statistical significance when compared between *Thuja oreintalis* treated and untreated animals of both genders (table 1).

Novel object

Novel object test trail 1 data analysis revealed that *Thuja oreintalis* treated male albino mice explored the test area better as they had explored objects A and B more than saline treated mice. In trial one, male animals treated with plant extract approached the object B significantly (P= 0.02) more than control group (table 2A). *Thuja oreintalis* treated male mice spend more time with novel object in trail II although they crossed the line less than control animals but none of the studied parameters reached statistical significance (table 2A, B). While saline treated female mice explored the objects A and B better than *Thuja oreintalis* treated animals in both trials but the difference in performance did not reached the statistical significance (table 2A, B).

DISCUSSION

As plants have the ability to synthesize a wide variety of chemical compounds that are involve in complex biological reactions in living systems, Hence, man is identifying and using medicinal plants throughout the human history (Christenhusz *et al*., 2011). Chemical compounds in plants mediate their effects on the human body through already known identical processes for the chemical compounds in drugs (Shiksharthi *et al*., 2011; Srivastava *et al*., 2012).

Thuja orientalis is also among the plants with known medicinal importance for several physiological abnormalities but little has been reported, until now, regarding its potential role in affecting the mammalian behavior. The present study was designed to determine the effect of 50mg/ml solvent/Kg boy weight of *Thuja orientalis* leaf extract on behavior, neuromuscular coordination, learning and memory formation of albino mice.

Rota rod test was conducted to check the motor coordination in the experimental treatments. Our results indicated gender specific effect of *Thuja orientalis*. It was observed that *Thuja orientalis* treated male albino mice showed better performance as they spent more time on rotating rod while female did not performed better as compared to saline treated mice indicating that *Thuja orientalis* has increased the neuromuscular coordination in male but adversely affects it in female albino mice ($P = 0.02$) (fig. 1). Our results are in agrrement to Lokesh *et al*. (2011) for female mice who had reported a dose dependent negative effect of *Thuja occidentalis* leaf extract on rota rod test performance in rats and mice.

Open field test was used to compare the locomotary and exploratory behavior in male and female albino mice following 50mg/Kg body weight *Thuja orientalis* plant extract supplementation. It was observed that leaf extract treated male albino mice performed better as they had covered more distance and has more mobile and immobile episodes indicating its better exploration as compared to saline treated mice, while treated female albino mice covered more distance but they remained mobile for very less time and had lesser mobile episodes than the saline treated mice indicating that the plant extract did not affect the locomotory and exploratory behavior of female albino mice. At the same time there was increased urination in treated females indicating the anxiolytic effect of leaf extract (Supplementary table 1). Our results are in agreement with Lokesh *et al*. (2011) who had also observed a dose dependent improve in exploratory behavior and anxiolytic effect in rodents following *Thuja occidentalis* leaf extract supplementation indicating that the two plant species has similar composition of leaf extract.

Bourin and Hascoet (2003) had experimentally demonstrated the anxiolytic and anxiogenic activity in mice with the help of light dark box test. The back-ground of this test was the fact that rodents avert brightly illuminated areas and spontaneous exploratory behavior is generally observed in response to mild stress or novel environment and light (Bourin and Hascoet, 2002). It was seen that *Thuja orientalis* treated male animals spent more time in dark, preferred its normal nocturnal behavior. When they reached the light area male mice began to move around the periphery and they acessed to transition window and reached in dark area indicating anxiogenic effect of *Thuja orientalis*. In case of female the result was opposite, here the saline treated female explored more in light and dark box because they had more transition frequency, spent less time in dark and more in light as compared to *Thuja orientalis* treated female albino mice that defecated and urinated more indicating their nervousness hence proved the anxiolytic effect of *Thuja orientalis* leaf extract but the difference in behavior did not reached statistical significance when compared with both male and female control animals (Supplementary table 2).

Lokesh *et al*. (2011) had demonstrated that when 100, 200 and 400 mg/Kg dose of *Thuja occidentalis* leaf extract was orally supplemented to rats and mice, their exploratory behavior improved in elevated plus maze test as their number of entries as well as duration of stay in open arms significantly increased as compared to non treated animals. Our results are in agreement with the above mentioned findings. When male and female albino mice were treated with 50mg/Kg *Thuja orientalis,* they covered more distance indicating better exploration and had more head dips showing more curiosity during elevated plus maze test as compared to control albino mice but statistically these results did not reached statistical significance (table 1).

Novel object test is used to demonstrate the recognition behavior in rodents. Results of novel object test in trial one indicated that leaf extract treated male mice showed better performance and explored objects A and B more than saline treated mice. Treated male mice showed its preference towards object B as its visits to object B very significantly ($P = 0.02$) more than the control group. In case of female the saline treated mice explored the arena better than *Thuja orientalis* treated female mice (table 2). In trial II the trend remained the same for saline treated female but *Thuja oreintalis* treated male mice showed its preference towards novel object and this reflects its better novel object recognition ability as compared to control mice but statistically this result did not reached the statistical significance.

CONCLUSION

We concluded that *Thuja orientalis* leaf extract has a potential to improve the various aspects of behavior in a gender specific manner in albino mice. It has improved the neuromuscular coordination, exploratory behavior and object recognition ability of young male mice during rota rod, open field, light dark box and novel object test. On the other hand, leaf extract has negatively affected the neuromuscular coordination and exploratory behavior of female albino mice, while the elevated plus maze test results revealed similar effects of *Thuja orientalis* leaf extract in both genders. The difference for various test performance between the treated and untreated group did not reached statistical significance, indicating that

probably the applied dose was too low to significantly impact the behavior of albino mice. Another important factor could be the fact that doses were not supplemented during neurological testing. The effects could be more pronounced if the oral dose would have been supplemented during the neurological testing and in higher concentration.

REFERENCES

Abbasi AM, Khan MA, Ahmad M and Zafar M (2011). Medicinal plant biodiversity of lesser Himalayas-pakistan. Springer London, pp.5-10.

Bourin M and Hascoet M (2003). The mouse light dark box. *Euro. J. Pharmacol*., **463**: 55-66.

Butlet MS (2004). The role of natural product chemistry in drug discovery. *J. Nat. Prod*., **67**: 2141-2153.

Christenhusz MJM, Reveal JL, FarjonA, Gardner MF, Mill RR and Chase MW (2011). A new classification and linear sequence of extant gymnosperms. *Magnolian Press England*, **19**: 55-70.

Edeoga HO, Okwu DE and Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol*., **4**: 685-688.

Iqbal S, Ali M, Akbar A and Iqbal F (2014). Effects of dietary Creatine supplementation for 8 weeks on neuromuscular coordination and learning in male albino mouse following neonatal hypoxic ischemic insult. *Neurolog Sci*., doi: 10.1007/s10072-014-2041-9

Lokesh D, Amitabha D, Sachin A and Avijeet J (2011). Neuropharmacological exploration of *Thujaoccidentalis* Linn. *Res. J. Pharmacol*., **2**(3): 143-148.

Mamidala E, Gujjeti RP (2013). Phytochemical and antimicrobial activity of Acmellapaniculata plant extracts. *J. Biol. Innov*., **2**(1): 17-22.

Owolabi J, Omogbai EKI and Obasuyi O (2007). Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigeliaafricana (Bignoniaceae) stem bark. *Afr. J. Biotechnol*., **6**(14): 882-885.

Saharan P, Duhan JS, Suresh KG and Surekha (2012). Antioxidental potential of various extracts of stem of Thujaorientalis *in vitro* study. *Int. J. App. Bio. Pharm. Technol*., **3**(4): 264-271.

Shiksharthi AR, Mittal S and Ramana J (2011). Systematic review of herbals as potential memory enhancers *I. J. R. P. B. S*., **2**(3): 19-28.

Shinwari ZK (2010). Medicinal plants research in Pakistan. *J. Med. Plant Res*., **4**(3): 161-176.

Srivasta P, Kumar P, Singh DK and Singh VK (2012). Biological properties of *Thuja Orientalis* Linn. *J. Adv. life Sci*., **2**(2): 17-20.

Sunyer B, Patil S, Frischer C, Hoger H, Selcher J, Brannath W and Lubec G (2007). Strain dependent effects of SGS742 in the mouse. *Behav. Brain Res*., **18**: 164-175.

Wendakoon C, Calderon P and Gagnon D (2012). Evaluation of selected medicinal plant extracted in different ethanol concentrations for antibacterial activity against human pathogens. *J. Med. Active. Pl*., **1**(2): 60-68.

Williams CJ, Aderhold D and Edyvean RGJ (1998). Comparison between biosorbents for the removal of metal ions from aqueous solutions. *Water Res.,* **32**(1): 216-224.

Zahra K, Khan MA and Iqbal F (2014). Oral supplementation of *Ocimumbasilicum* enhances the locomotory, exploratory and learning behavior of adult male albino mice. *Neurologl Sci*., 10.1007/s10072-014-1913-3014-1913-3.