REPORT

Ethanolic *Ficus carica* leaf extract supplementation affects the behavior of male albino mice

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Abstract: *Ficus carica* has been known for its medicinal benefits for the treatment of metabolic, cardiovascular and respiratory problems but little information is available regarding the effects of its leaf extracts on behavior. Present study was designed to report the effect of *Ficus carica* leaf extract on the selected aspects of male albino mouse behavior. Six week old male albino mice were orally treated for 14 days either with 100 mg/ml solvent/ Kg body weight of *Ficus carica* leaf extract or with commercially available saline solution (Otsuka, Pakistan). A battery of neurological tests (Morris Water Maze, Elevated Plus maze, Novel object test) were applied in order to determine the effect of *Ficus carica* extract on exploratory behaviour, learning and memory in male albino mice. It was observed that oral supplementation of 100mg/ ml solvent/Kg body weight of *Ficus carica* leaf extract for 2 weeks enhanced neuromuscular co-ordination as they had significantly higher mean speed ($P = 0.001$) than control group during elevated plus maze test. Morris water maze and novel object test performance remained unaffected ($P > 0.05$) when compared between *Ficus carica* leaf extract treated and untreated mice.

Keywords: *Ficus carica*, Morris water maze, elevated plus maze, novel object, Learning and memory.

INTRODUCTION

In flowering plants *Ficus* constitutes one of the largest genera (Ronsted *et al*., 2008). The *Ficus carica* (fig) plant traditionally has been used to treat various ailments such as inflammation, gastric problems and cancer (Hamed, 2011). Majority research work on the biological activities of the plant are mainly conducted on its crude extracts which have been proven to exhibit many biological activities such as anticancer, hepatoprotective, hypoglycemic, hypolipidemic and antimicrobial activities (Mawa *et al*., 2013). Leaves and fruits of the plant are used in phytochemical studies and are enrich in organic acids, phenolics and volatile compounds (Joseph and Raj, 2011). Analysis of hexane extract by using phytochemical screening confirmed the presence of flavonoids (quercetin) which have been associated with central nervous system (CNS) active moiety (Vasundhara *et al*., 2013). Little information is available regarding the effects of *Ficus carica* leaf extracts on behavior in albino mice. The aim of the present study was to demonstrate the effect of *Ficus carica* leaf extracts on muscular activity, learning and spatial memory in male albino mice.

MATERIAL AND METHOD

For present study six weeks old, male albino mice were used. Punjab University Lahore Department of Zoology, Quaid-i-Azam campus donated breeding pairs of albino mice. The breeding colony of albino mice was maintained at the animal facility in Bio Park of Bahauddin Zakariya University Multan, Pakistan.

Locally manufactured small rodent cages were used to keep the animals which filled with wood chips. Standard mouse diet and water were available *ad libitum* in breeding colony. Room temperature was monitored at 22 ± 1°C with light/dark rhythm at 14:10 hours. The room was provided with an artificial light (200 Watt) from 8 a.m. to 10 p.m. Ethical committee of Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan approved all the experimental procedures.

Ficus carica's leaves extract preparation

Ficus carica leaves were weighed and dried in shade for seven days for the preparation of extract. Dried leaves were powered by using Blender. Powdered form of *Ficus carica*, were soaked in for 15 days in 70 % aqueous-methanol (v\v) and 30% distilled water using amber colored glass bottles with occasional 3-4 times shaking in a day. After every shake opened the lid of bottle for a moment to remove the gas and tied the lid again. To remove the vegetative debris from the soaked material double layered muslin cloth was used to filter. The filtered fluids were subsequently again filtered through filter paper (Zahra *et al*., 2015). The whole procedure was repeated twice with residues. The filtrates were evaporated on a rotator evaporator (BUCHI, Switzerland) under reduced pressure (-760 mm of Hg) at 37 C to a thick, semi-solid paste. The thick pastes were put in Petri-

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plates for further evaporation. Concentrated extracts were stored at 4^{o}C until tested and utilized.

Experimental design

Stock solution of *Ficus carica* leaf extract was prepared by dissolving 100 mg plant extract in 1ml of distilled water. Two groups of male albino mouse (N = 10 for both) orally treated with 100ml/ml solvent/Kg body weight of *Ficus carica* leaf extract or with commercially available saline solution (Otsuka, Pakistan) for 14 days. Each animal was weighted before treatment every day.

Dose was also applied during neurological testing. A battery of neurological tests (Morris water maze, Elevated plus maze, Novel object test) were applied in order to determine the effect of *Ficus carica* extract on exploratory behavior, learning and memory in male albino mouse.

Morris water maze (MWM)

Morris water maze test apparatus consisted of a circular pool with diameter of 122 cm and depth of 76 cm. A hidden platform which is 1.5 cm beneath water surface was used to train the mice to escape from water.

The location of a hidden platform can only be recognized by using distal extra maze cues attached to the room walls. MWM was attached with a computerized tracking system (Any maze, Stoelting, USA) coupled with camara (XPod-058, China). The pool was divided into four quadrants by using compass locations: NE, NW, SW and SE. The hidden platform location was always in the middle of NE quadrant during the whole experiment. The whole experiment was performed by using the protocol followed by Gillani *et al*. (2014).

Swim strategies

Janus, (2004) described seven different types of swimming behaviors strategies during training of MWM. These strategies are classified as wall hugging, chaining, random and scanning at the start of training trials. Focal search and direct swimming showed the learning and memory formation during trials. These strategies can be described as spatial strategies (direct and focal) and non spatial strategies (Scanning, random, focal incorrect) (Gillani *et al*., 2014).

Elevated plus maze

Elevated Plus Maze was made of a black Plexiglas floor with a 5 x 5 cm central square platform from which two 45 x 5 cm open arms radiated with 0.25 cm high edges and two 45 x 5 cm closed arms with 40 cm high walls. Mice were placed in the central square of Plus-maze facing an open arm and were then allowed to explore the apparatus for 5 minutes. A video camcorder placed above the center of the maze recorded behavior. Head-dips, urination and defection were recorded by experimenter, arm entries and time in each arm were recorded with the Limelight video camera (Addlink, Barcelona) based tracking system (Anymaze, USA) following Riaz *et al*. (2017).

Novel object test

Memory formation without the help of spatial cues can be tested by novel object recognition.

The animal was trained for two trials each of 5 minutes with the interval of 20 minutes to recognize certain objects in an area of 40 cm x 40 cm log with 70 cm high walls. After training the animal was tested by replacing one of the old objects with a novel object. 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 while in second trial, 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 following Riaz *et al*. (2017).

STATISTICAL ANALYSIS

Data was expressed as mean ± standard error (SE) of mean by using statistical package Minitab (Version 16, USA). Morris water maze, Elevated plus maze and Novel object test between *Ficus carica* and saline treated male albino mice were compared by using 2 sample t-test.

RESULTS

Elevated plus maze test

Analysis of the results revealed that during the elevated plus maze test the performance of *Ficus carica* treated male mice was better as they covered more distance with more speed, had more head dips to explore and were immobile for less time than saline treated male albino mice. The difference in the performance did not reach the statistical difference for any of the studied parameters except mean speed that was significantly higher (P = 0.001) in *Ficus carica* treated males than saline treated male albino mice (table 1).

Novel object test

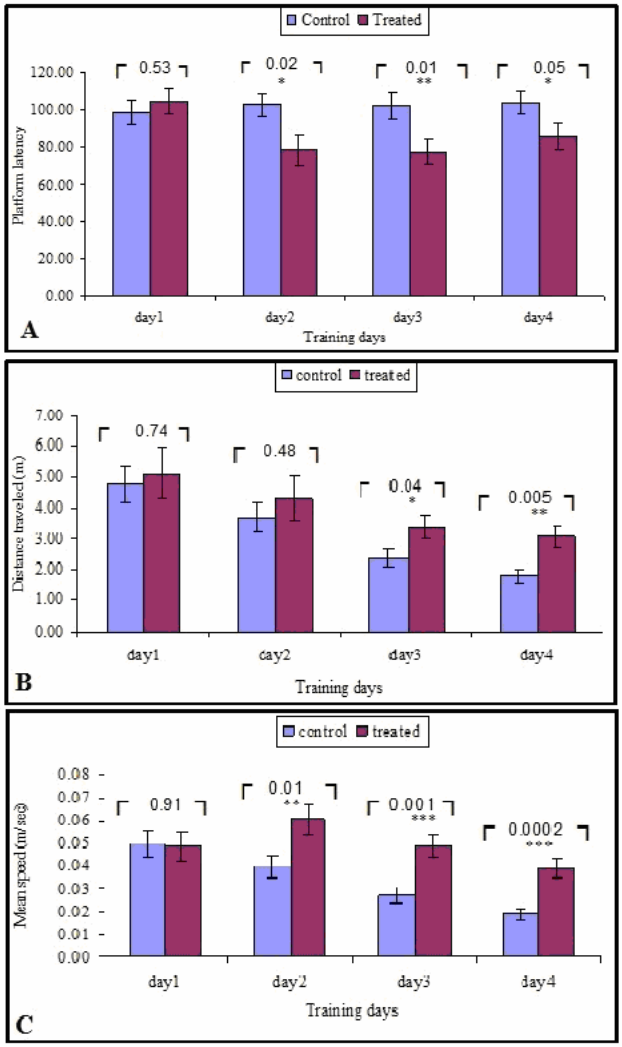
Analysis of results from first novel object trial indicated that *Ficus carica* treated male mice had more line crossing and they approached object B more frequently while control animals approached object A and spent more time with both objects than plant extract treated male albino mice but the difference in performance did not reach the statistical significance for any parameter (table 2A).

In novel object trail 2, *Ficus carica* treated mice showed better performance again as they had more line crossings and also approached both novel and old object more

frequently and spent more time with them than saline treated controls but again the difference in performance did not reached statistical significance for any of the studied parameter (table 2B).

Morris water maze test (Acquisition phase)

Analysis of the results indicated that *Ficus carica* treated male albino mice showed gradual improvement in memory formation as their latency to reach platform decreased with increasing training sessions. *Ficus carica* treated animals had significantly lower latency on training day 2 (P = 0.02), 3 (P = 0.01) and 4 (P = 0.05) than saline treated control group (fig. 1A).

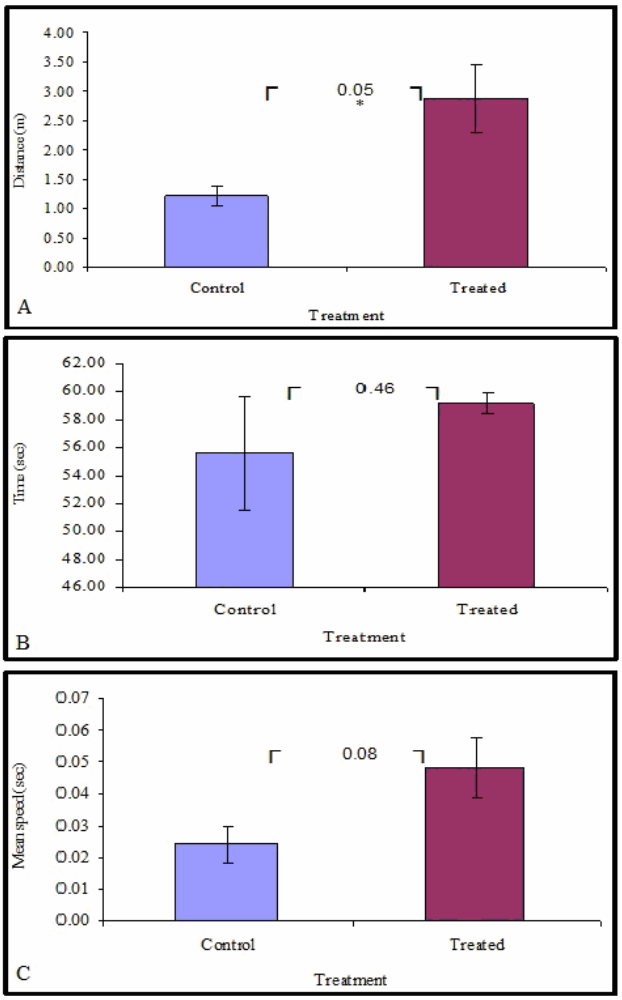


P>0.05= Non significant; P< 0.05= least significant (*); P< 0.01= significant (**); P< 0.001= Highly significant (***)

Fig. 1: Comparison of latency (A), total distance (m) travelled (B) and swimming speed (m/sec) to reach platform (C) between *Ficus carica* (100mg/ml of solvent/ Kg body weight) treated and untreated male albino mice during acquisition phase of Morris Water Maze test. Data is expressed as mean ± standard error of mean. P-value indicates the results of 2 sample t tests.

Although the time taken by *Ficus carica* treated male albino mice was less than control to reach the platform but results indicated that they covered more distance than control to reach platform and this difference was significant on training day 3 (P=0.04) and 4 (P=0.005) (fig. 1B).

This finding that *Ficus carica* treated males covered more distance in less time to reach platform can be justified by the observation that *Ficus carica* treatment had significantly higher mean swimming speed than control animals during training day 2 (P = 0.01), 3 (P = 0.001) and 4 (P = 0.002) (fig. 1C).



P>0.05= Non significant; P< 0.05= least significant (*)

Fig. 2: Comparison of the distance (A), time (B) and mean speed (C) to reach the platform area between *Ficus carica* (100mg/ml of solvent/Kg body weight) treated and untreated male albino mice for 6 weeks in Morris Water Maze test during probe trail test. Data is expressed as mean ± standard error of mean. P-value indicates the results of 2 sample t tests.

Morris water maze test (Probe trial)

Results of probe trial indicated that *Ficus Carica* treatment had no significant effect on learning and

Table 1: Comparison of various studied parameters between *Ficus carica* (100 mg/ml of solvent/ Kg body weight) and saline treated adult male albino mice during elevated plus maze test. All values are expressed as mean ± standard error. P-value indicates the result of two sample t-test.

Parameters	Saline treated control (N = 10)	*Ficus Carica* treated (N = 10)	P- Value
Distance (m)	11.12 ± 1	12.63 ± 0.54	0.2
Mean Speed (m/s)	0.03 ± 0.03	0.04 ± 0.01	0.001***
Time mobile (sec)	211.5 ± 13	235.7 ± 9.2	0.2
Time immobile (sec)	88.5 ± 13	64.3 ± 9.2	0.2
Mobile episodes	20.17 ± 2.8	17.17 ± 1	0.4
Immobile episodes	19.33 ± 2.9	16.33 ± 1	0.4
Rotations	12.5 ± 0.96	14.33 ± 0.71	0.2
Clockwise rotations	6.83 ± 0.79	8 ± 0.68	0.3
Anti clockwise rotation	5.67 ± 0.92	6.33 ± 0.33	0.2
Urination	0	0	0
Defecation	1.67 ± 0.49	0.67 ± 0.49	0.12
Head dipping	7.67 ± 2	10.50 ± 3.9	0.5

P > 0.05 = Non significant**;** P < 0.001 = Highly Significant (***)

Table 2A: Comparison of various studied parameters between *Ficus carica* (100mg/ml of solvent/ Kg body weight) and saline treated adult male albino mice during first trial of Novel object test. All values are expressed as mean ± standard error.

Parameters	Saline treated control (N = 10)	*Ficus carica* treated (N = 10)	P-value
Line cross	22 ± 3.7	23.8 ± 5.1	0.8
Stretch attend reflex	1.83 ± 0.79	4.83 ± 2	0.2
Approaches object A	19.2 ± 5.6	9.33 ± 2.7	0.2
Approaches object B	8.67 ± 1.6	8.5 ± 1.1	0.9
Time object A (sec)	56.5 ± 9.7	41 ± 9.1	0.3
Time object B (sec)	54.5 ± 7	61.2 ± 16	0.7

P > 0.05 = Non significant

memory formation as they took more time (P=0.46), covered more distance (P=0.05), although with higher swimming speed (P=0.08). *Ficus carica* treated animals visited platform area more frequently than saline treated male albino mice but the difference did not reach the statistical significance (P = 0.55) (fig 2A-C).

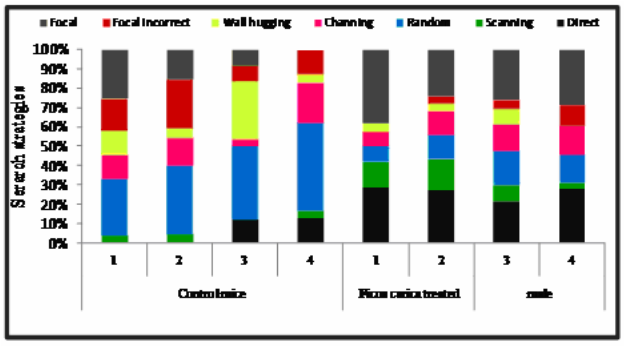


Fig. 3: Comparison of swimming strategies during acquisition phase of Morris Water Maze between *Ficus carica* (100 mg/ml of solvent/ Kg body weight) treated and untreated male albino mice.

Swimming strategies

Comparison of swimming strategies indicated that male albino mice treated with *Ficus carica* showed better learning during acquisition phase as their direct approaches to platform and focal search behaviour was improving over time while saline treated males had decreased direct and scanning strategies while random focal incorrect and chaining behaviour increased over time indicating poor learning (fig. 3).

DISCUSSION

Ficus carica is a deciduous tree which grows in tropical and subtropical regions of Pakistan and commonly known as fig tree. In traditional medicines leaves of *Ficus carica* are used in treatment of diabetes, skin, ulcer and diarrhea (Imran *et al*., 2011). The present project was designed to determine the effect of 100mg/ml solvent/ Kg body weight of *Ficus carica* leaf extract on selected aspects of behaviour in male albino mice.

Analysis of the results obtained by Morris water maze test indicated that *Ficus carica* treated male albino mice showed gradual improvement in memory formation as their latency to reach platform decreased with increasing training session (fig. 1). It was also observed that leaf extract treated animals covered more distance than controls. This finding that *Ficus carica* treated males covered more distance in less time to reach platform can

Table 2B: Comparison of various studied parameters between *Ficus Carica* (100mg/ml of solvent/ Kg body weight) and saline treated adult male albino mice during second trial of Novel object test. All values are expressed as mean ± standard error.

Parameters	Saline treated Control (N = 10)	*Ficus carica* treated (N = 10)	P-value
Line cross	13.17 ± 3.1	15 ± 3.7	0.7
Stretch attend reflex	2.33 ± 0.8	2.17 ± 1.1	0.9
Approaches Old Object	5.17 ± 1.1	6 ± 1.4	0.3
Approaches Novel object	5.67 ± 0.8	7.83 ± 1.5	0.2
Time Old object (sec)	31 ± 13	6 ± 15	0.7
Time Novel object (sec)	54 ± 4.9	61.2 ± 11.1	0.7

P > 0.05 = Non significant

be justified by the observation that *Ficus carica* treatment had significantly increased mean swimming speed than control animals during training days (fig. 1). This observation was further supported by the fact that *Ficus carica* treated male albino mice remained immobile for less time than saline treated control males and actively covered long distance in less time but the difference between the two treatments for this parameter did not reach statistical significance (P=0.46). These results indicated that the leaf extract of *Ficus carica* has no significant effect on learning in Morris water maze task. On the other hand the improved mean swimming speed and greater distance covered in less time by *Ficus carica* treated male albino mice indicated that the plant extract has affected the neuromuscular co-ordination of the male albino mice. Our results are not in accordance with Vasundhara *et al*. (2013) who had reported the memory enhancing effects of hexane extract of *Ficus carica* leaves. One of the possible reasons for contradictory reason could be the different dose used by us (100mg/ml/ Kg body weight) then that study (200 mg/ml/Kg body weight) along with different extraction methods used in two studies that might have affected the composition of the extracts.

The standard elevated plus-maze test is commonly used to assess anxiety and exploratory behavior in laboratory animals (Rat/ mice) (Hogg, 1996). The data analysis from elevated plus maze test indicated that the *Ficus carica* leaf extract treated male albino mice performed better as they covered more distance (P = 0.23) with more head dips (P = 0.53) to explore and remained immobile for less time (P = 0.16) than the control group but this difference in performance did not reach the statistical significance for any studied parameter except the mean speed which indicates that the plant extract improved the muscular activity/neuromuscular co-ordination in male albino mice (table 1), complementing our Morris water maze observations (table 3).

Novel object test results indicated that the *Ficus carica* treated male albino mice showed better performance in the second trial as they had more line crossings (P = 0.71) and also approached both novel (P = 0.24) and old object (P = 0.25) and spent more time with them than saline treated male albino mice but the difference in performance was not statistically significant for any studied parameter (tables 2-3).

In conclusion, we have observed that 100 mg/ ml solvent / Kg body weight of *Ficus carica* leaf extract has a potential to improve muscular activity, exploratory behaviour, recognition ability and spatial memory but probably the dose concentration was too low to generate significant effects. We recommend similar studies with higher *Ficus carica* leaf extract dose concentrations to get a complete picture regarding the effects of this extract on male albino mice behaviour.

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