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Research Article

ACUTE & SUB CHRONIC TOXICITY STUDIES AND PHARMACOLOGICAL EVALUATION OF *FICUS BENGALENSIS* L. (FAMILY: MORACEAE) ON SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT IN EXPERIMENTAL ANIMALS

Phool Chandra[1*], Neetu Sachan[1], Aakash Chaudhary[1], Mayank Yadav[2], Kamal Kishore[3], Ashoke Kumar Ghosh[1]

[1]School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Delhi Road (NH-24), Moradabad-244 102 (UP), India
[2]Adarsh Vijendra Institute of Pharmaceutical Sciences (AVIPS), Gangoh, Saharanpur-247 341 (UP), India
[3]Department of Pharmacy, MJP Rohilkhand University, Bareilly-243 006 (UP), India

***Corresponding author**
Phool Chandra
School of Pharmaceutical Sciences
IFTM University
Lodhipur Rajput, Delhi Road (NH-24)
Moradabad-244 102 (UP), India
Phone No: +91 591 2360022
Fax: +91 591 2360818
Email: chandraphool@yahoo.co.in

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Abstract

Aim of the research were to investigate acute, sub-chronic oral toxicities of aqueous extract from the bark of *Ficus benghalensis* (AFB) in rats and its effect on cognitive parameters of young and aged mice. Acute toxicity study was done with 5000 mg/kg of aqueous extract from the bark of *Ficus benghalensis* and in sub-chronic oral toxicity study; evaluations were carried out after administering extract (500 and 1000 mg/kg body wt, p.o., daily) for 28 days to the rats. Anti-amnesic activity of the AFB was evaluated using passive avoidance test and the plus maze tests in scopolamine-induced (1 mg/kg body weight, i.p.) amnesia and old aged mice. LD50 may be greater than 5000 mg/kg (orally) for AFB. On 28 days sub chronic oral administration (500 and 1000 mg/kg of extract) in rats no signs of toxicity were observed. Oral treatment of mice separately with AFB (150 and 300 mg/kg body weight) and vitamin C (250 mg/kg body weight) significantly mitigated scopolamine-induced memory deficits in passive avoidance test. In the plus maze test, oral treatment of extract and vitamin C significantly ameliorated scopolamine-induced memory deficits in young mice as well as ameliorate age owned memory deficits in old aged mice showing the formation of spatial memory. Results suggest that AFB has no signs of toxicity at 5000 mg/kg body weight of rats orally; sub-chronically and exerts *in vivo* antiamnesic activity. The *Ficus benghalensis* might offer a useful therapeutic choice in the treatment of Alzheimer's disease.

Keywords: *Ficus benghalensis*, Moraceae, Toxicity, Scopolamine, Amnesia.

INTRODUCTION

Ficus benghalensis (Moraceae, Mulberry family) is commonly known as Banyan tree or Vata or Vada tree in Ayurveda. There are more than 800 species and 2000 varieties of *Ficus* species, most of which are native to the old World tropics. *Ficus benghalensis* a remarkable tree of India sends down its branches and great number of shoots, which take root and become new trunk. This tree is considered to be sacred in many places in India. Earlier glucoside, 20-tetratriaconthene-2-one, 6-heptatriacontene-

10-one, pentatriacontan-5-one, beta sitostirol-alpha-D-glucose and meso-inositol have been isolated from the bark of *Ficus benghalensis (Subramanian et al., 1978 and The wealth of India, 2005)*. Leaves contain crude protein 9.63%, crude Þbres-26.84%, CaO-2.53%, and Phosphorus-0.4%. It yields latex containing Caoytchoue (2.4%), Resin, Albumin, Cerin, Sugar and Malic acid. It is used in Ayurveda for the treatment of Diarrhea, Dysentery and piles *(Mukherjee et al., 1998 and Husain et al., 1992)*, teeth disorders *(Aiyer et al., 1957)*, Rheumatism, skin disorders like sores *(Warrier et al., 1993)*, To boost immune system *(Gabhe et al., 2006)*, as a hypoglycemic *(Shrotri et al., 1960, Deshmukh et al., 1960, Augusti et al., 1975 and Augusti et al., 1994)*. The extracts of *Ficus benghalensis* were also reported to inhibit insulinase activity from liver and kidney *(Achrekar et al., 1991)*. Fruit extracts exhibited anti-tumor activity in the potato disc bioassay *(Mousa et al., 1994)*. Two ßavonoid compounds, viz. 5, 7-dimethyl ether of leucopelargonidin 3-0-alpha-L rhamnoside and 5, 3'-dimethyl ether of leucocyanidin 3-0-alpha-D galactosyl cellobioside were obtained from the bark of *F. benghalensis* evaluated for anti-oxidant activity in hyperlipidemic rats *(Daniel et al., 1996)*. It was also found to inhibit the lipid peroxidation *(Shukla et al., 2004)*. Various extracts of *Ficus benghalensis* was screened for its anti-allergic and anti-stress potential in asthma by milk induced leucocytosis and milk induced eosinophilia *(Taur et al., 2007)*. Other species of Ficus viz. *Ficus inspida, Ficus carica (De-Amorin et al., 1999), Ficus religiosa (*Iqbal et al., 2001*)* was found to be reported to have anthelmintic activity. Based on this, an attempt has been made to evaluate the anthelmintic potency of *Ficus benghalensis*.

Alzheimer's disease (AD) is the most common neurodegenerative disease, leading to dementia characterized by a progressive decline of cognitive function due to degeneration of the cholinergic nervous system *(Lee et al., 2006).* Similarities in the memory impairments between Alzheimer patients and scopolamine-treated animals have been reported, and it has been proposed that scopolamine, a muscarinic cholinergic receptor antagonist, could serve as a useful pharmacological tool to produce a partial model of the disorder *(Bartus, 2000).* Recent studies have pointed out that AD is associated with inflammatory processes. Reactive oxidative species (ROS) are able to damage cellular constituents and act as secondary messenger in inflammation. The use of antioxidants may be useful in the treatment of AD *(Gilgun-Sherki et al., 2002).* Many studies suggest that supplementation with antioxidants may delay the development of Alzhemier's disease *(Stocker, 1994)*. Present study was aimed to evaluate the oral acute and sub-chronic toxicity of the aqueous extract from the bark of *Ficus benghalensis* L. (AFB) in rats with the hope that the results would provide information on the safety of this extract prior to the evaluation of its therapeutic efficacy in humans. Also, effects of AFB on cognitive parameters of young and aged mice were evaluated.

MATERIALS AND METHODS

Collection of Plant materials: The Bark of *Ficus bengalensis* were collected in February 2011, from the local place of Lodhipur, Moradabad UP (India). The plant materials were taxonomically identified and authenticated by Dr. (Mrs) Beena Kumari (Taxonomist) at Hindu College, Moradabad UP (India) with reference number: HC.MBD/HAP/BK/2011/4/333.

Extraction: The technique for separation of chemical of active substance from crude drug is called extraction *(Kokate et al., 2006)*. Dried barks were pulverized to fine powder and that was passed through 20 mesh size. Powder was soaked with distilled water for 24 hrs. Then it was filtered with cloth and filtrate was concentrated. Semi-solid mass obtained was further concentrated by vacuum drying to

yield a solid residue. This was kept in refrigerator for preliminary phytochemical and pharmacological screening.

Preliminary phytochemical studies

AFB obtained was subjected to preliminary qualitative tests for various plant constituents by suitable chemical tests *(Khandelwal, 2011; Trease and Evans, 1987)*.

Animals: Swiss albino male mice, weighing 22-28 g (young), 8 weeks of age and 30-35g (old) and Wistar albino rats (150-200g) were obtained from animal house of College of Pharmacy, IFTM, Moradabad (India). Animals were housed 5 per cage, allowed access to water and food ad *libitum* and maintained in a constant temperature 22±2^{0}C and humidity 50-55% and 12 h of light/dark cycle. All experimental procedures were carried out in accordance with the CPCSEA Guidelines and were approved by the Institutional Animal Ethical Committee at College of Pharmacy, IFTM, Moradabad (India).

Acute toxicity study: The highest attainable dose AFB (5000 mg/kg) was used in Organization for Economic Cooperation Development *(OECD, 2001)* guideline 423. Three female rats, each sequentially dosed at intervals of 48 hrs, were used for the test. Once daily cage side observations included changes in skin, fur, eyes, mucous membrane (nasal), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks.

Sub-chronic oral toxicity study in rats: Sub-chronic oral toxicity study in rats was carried out Organization for Economic Cooperation Development *(OECD, 1995)* guideline 407. Healthy Wistar albino rats (3-month old, 150–200 g BW) were randomly assigned to each of three groups of 10 rats (5 females and 5 males). The AFB, dissolved in distilled water, was administered by daily gavage for 28 days, to groups I to III (Control, 500, 1000mg/kg, respectively). The animals were observed for signs of toxicity and mortality throughout the experimental period. The BW, water and food consumption were recorded weekly. At the end of the 28-day experiment, the animals, fasted for 12 hrs, were sacrificed by over dose of ether. Blood was collected into two tubes of which tube one containing EDTA was processed immediately for haematological parameters while other tube without additive was centrifuged at 3000×g at 4^{0}C for 10 min to obtain serum. The organs (kidneys, liver, lungs, heart, testes, spleen and uterus) were weighted.

Blood Analysis: The hematological parameters including white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (HB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT) count using autoanalizer.

Biochemical Analysis: Serum bio-chemistry parameters including alanine amino transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (g/dl), albumin (g/dl), bilirubin direct (mg/dl), total bilirubin (mg/dl).

Experimental procedure for study on cognitive parameters

For this experiments young and aged mice were randomly distributed into four groups each (n=8). In the aged mice category, first group received only vehicle treatment perorally for a period of 7 days. Subsequent two groups of animal received varying doses of AFB (150 and 300 mg/kg p.o. once a day for a period of 7 days. The last group received vitamin C (250 mg/kg, p.o.) as a standard drug. Similarly, in young category mice, first group received only vehicle treatment perorally for a period of 7 days. Subsequent two groups of animal received varying doses of AFB (150 and 300 mg/kg p.o. once a day for a period of 7 days. The last group received vitamin C (250 mg/kg, p.o.) as a standard drug. In young

animals, amnesia was induced by administration of scopolamine (1 mg/kg, i.p.) after 60 min of administration of doses on 7th day and the step down latency (SDL) and Transfer latency (TL) recorded after 30 minute. After 24 hrs, animals tested for their retention using passive avoidance and elevated plus maze task.

Elevated plus maze test: An elevated plus maze consisting of two open arms (35×6 cm) and two enclosed arms (35×6×15 cm) was used. The maze was elevated to the height of 25 cm. Mice were placed individually at the end of an open arm facing away from the central platform and the time it took to move from the end of open arm to either of the closed arms (transfer latency, TL) was recorded *(Chintawar et al., 2002)*.

Passive avoidance test: Passive-avoidance task is a method widely used for screening drugs affecting learning and memory. Passive avoidance test was assessed using passive shock avoidance paradigm as previously used by (Kulkarni and Verma 1992). The apparatus consisted of an electric grid (24×/24 cm) with an elevated platform (8×/8×0.5 cm) in the centre of the grid. The mouse was placed on the elevated platform, i.e. the shock free zone (SFZ), and the step down latency (SDL) was noted. Immediately after stepping down from the SFZ, mouse received an electric shock (2mA) of 2s duration through the grid floor and withdrawn and placed in their cages.

Locomotor activity test: The animal locomotor behavior was assessed using actophotometer, described by (Kulkarni 1998). Before subjecting the animals to cognitive tasks, they were individually placed in actophotometer and the ambulatory activity registered for five-minute period. The locomotor activity was expressed in terms of total photobeam count 5 min per animal.

STATISTICAL ANALYSIS

All the experimental results were expressed as mean±S.E.M. Data were analyzed by analysis of variance (ANOVA), followed by Dunnett's test with the level of significance set at $P<0.05$.

RESULTS

Preliminary phytochemical studies

The preliminary phytochemical analysis of AFB revealed the presence of compound listed in the table 1.

Table 1 Phytochemical Screening of the AFB

S.No	Constituents	Tests	Observation
1.	Carbohydrates	Molish's test	+
		Fehling's test	+
		Barfoed's test	+
2.	Proteins	Millon's Test	+
		Biuret Test	+
3.	Amino acids	Ninhydrin Test	+
		Millon's Test	+
4.	Fixed oils and Fats	Saponification Test	-
5.	Saponins	Foam Test	+
6.	Phenolics and Tannins	$Fecl_3$ Test	+
		Gelatin Test	+
		Lead Acetate Test	+
7.	Alkaloids	Dragondroff's Test	+
		Mayer's test	+
		Hager'stest	+
		Wagner's Test	+
8.	Flavonoids	Aqueous NaOH Test	+
		Conc. H_2SO_4 test	+
		Shinoda's test	-

+ = present, - = absent

Acute toxicity study: In acute toxicity study, AFB at a dose of 5000 mg/kg caused neither visible signs of toxicity nor mortality. Generally, the reduction in body weight gain and internal organ weights is a simple and sensitive index of toxicity after exposure to toxic substance. Furthermore, gross examination of internal organs of all female rats revealed no detectable abnormalities. Thus, it can be concluded that AFB is virtually nontoxic.

Sub-chronic oral toxicity study in rats

Body and organ weights: As shown in figure 1, both control and animals treated with AFB presented constant increase in body weight. Animal receiving AFB (1000 mg/kg) showed decrease in body weight at the fourth week of treatment, as compared to control rats. Macroscopic analysis of target organs of treated animals (liver, heart, lung, kidney and

spleen) did not show significant changes in colour and texture when compared with the control group. Relative weights of organs were not significantly affected by AFB treatment (Figure 2).

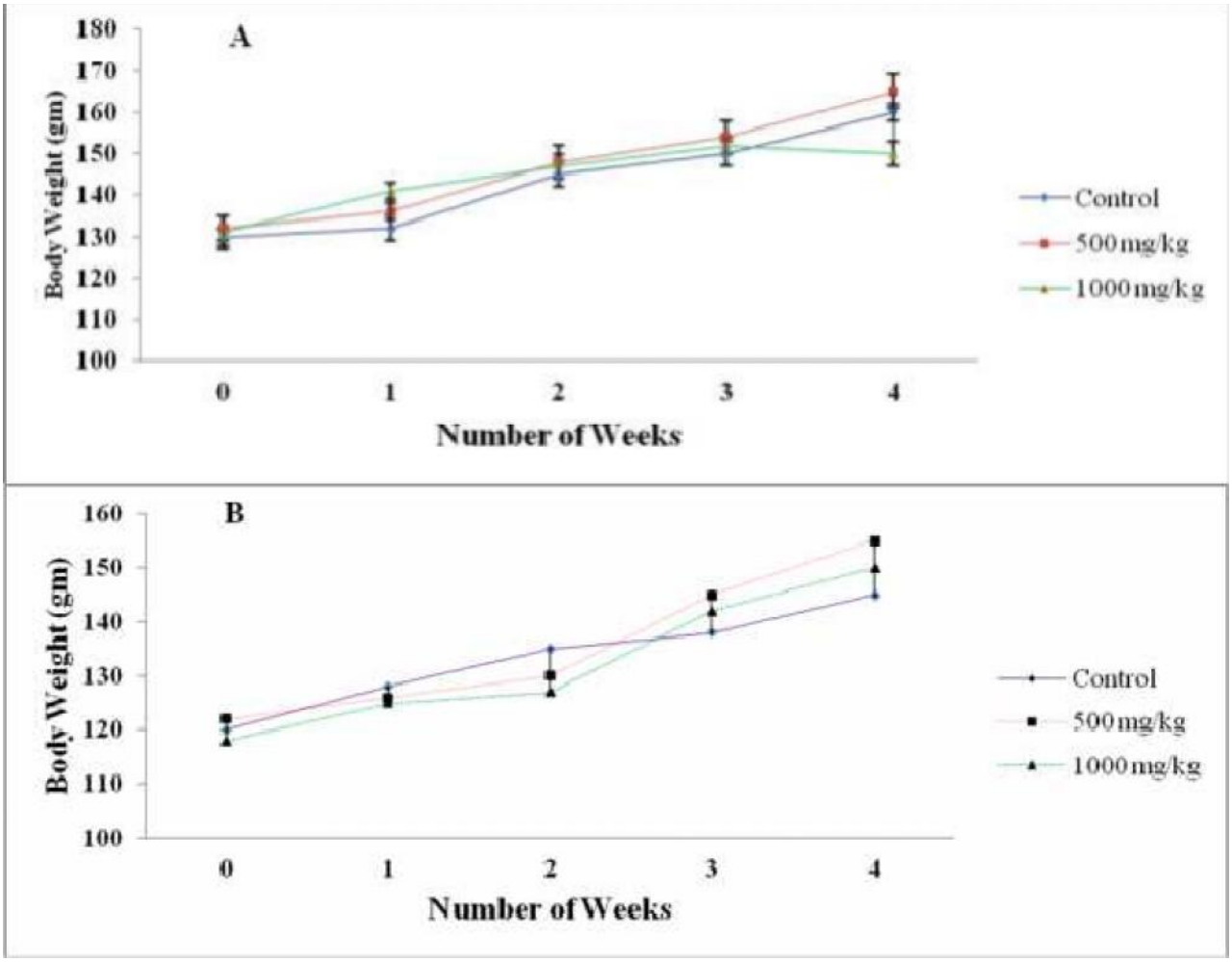

Figure 1 Body weight gain in rats treated orally with control, aqueous extract of bark of *Ficus benghalensis* (AFB 500 & 1000 mg/kg) for 28 days. Results are mean±SEM. ANOVA, $p>0.05$ between groups in the same day. **A** for male rats and **B** for female rats.

Blood Analysis: The status of bone marrow activity and intravascular effects were monitored by hematological examination as summarized in tables 2 and 3. In male group, the red blood cells (RBC) and hemoglobin (HGB) were significantly increased whereas the mean corpuscular volume (MCV) was increased in female rats.

Biochemical Analysis: Functioning of liver were assayed by studing the Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Albumin, Bilirubin and Direct bilirubin and are presented in table 4 & 5.

Effects on memory impairment

Elevated plus maze test: AFB 150 and AFB 300 treated mice shows significant decreases in retention time in comparison to vehicle treated mice and the result are statistically significant ($p<0.01$) which also comparable to the mice treated with vit C (Figure 3).

Table 2: Hematological values of male rats in sub chronic toxicity of the aqueous extract from the bark of *F. bengalensis*

Parameters	Control	500mg/kg	1000mg/kg
WBC (×103/µL)	7.40 ± 0.06	7.40 ± 0.35	7.16 ± 0.25*
RBC (×106/µL)	7.10 ± 0.02	7.73 ± 0.04	7.92 ± 0.14
HGB (g/dl)	13.25 ± 0.2	13.20 ± 0.51	13.1 ± 0.6
HCT (%)	42.10 ± 0.41	43.18 ± 0.43	44.56 ± 0.52
MCV (fl)	59.30 ± 0.19	55.88 ± 0.72	56.27 ± 1.12
MCH (pg)	18.66 ± 0.12	17.11 ± 0.73	16.59 ± 0.83
MCHC (g/dl)	31.47 ± 0.23	30.62 ± 1.10	29.49 ± 1.17
Platelets(×103/µL)	830 ± 4.24	819.2 ± 4.30	845.4 ± 6.44

Values are expressed as mean±S.E.M., n=5, control group were treated with vehicle and others groups two groups were treated daily with aqueous extract from the bark of *F. bengalensis* (500 & 1000mg/kg) for 28 days. * Significantly different from control, $p < 0.05$.

Table 3: Hematological values of female rats in sub chronic toxicity of the aqueous extract from the bark of *F. bengalensis*

Parameters	Control	500mg/kg	1000mg/kg
WBC (×103/µL)	7.32 ± 0.06	7.32 ± 0.43	7.22 ± 0.53
RBC (×106/µL)	6.70 ± 0.02	7.38 ± 0.43*	7.44 ± 0.51*
HGB (g/dl)	13.15 ± 0.2	13.50 ± 0.54	13.20 ± 0.61
HCT (%)	40.10 ± 0.41	43.40 ± 2.54	43.40 ± 0.57
MCV (fl)	59.85 ± 0.19	58.85 ± 6.13	58.38 ± 4.60
MCH (pg)	19.63 ± 0.12	18.24 ± 1.19	17.75 ± 1.22
MCHC (g/dl)	32.79 ± 0.23	31.02 ± 1.68	30.41 ± 1.47
Platelets(×103/µL)	836± 3.31	830.0 ±4.98	850.2 ± 5.33

Values are expressed as mean±S.E.M., n=5, control group were treated with vehicle and others groups two groups were treated daily with aqueous AFB (500 & 1000mg/kg/day) for 28 days. * Significantly different from control, $p<0.05$.

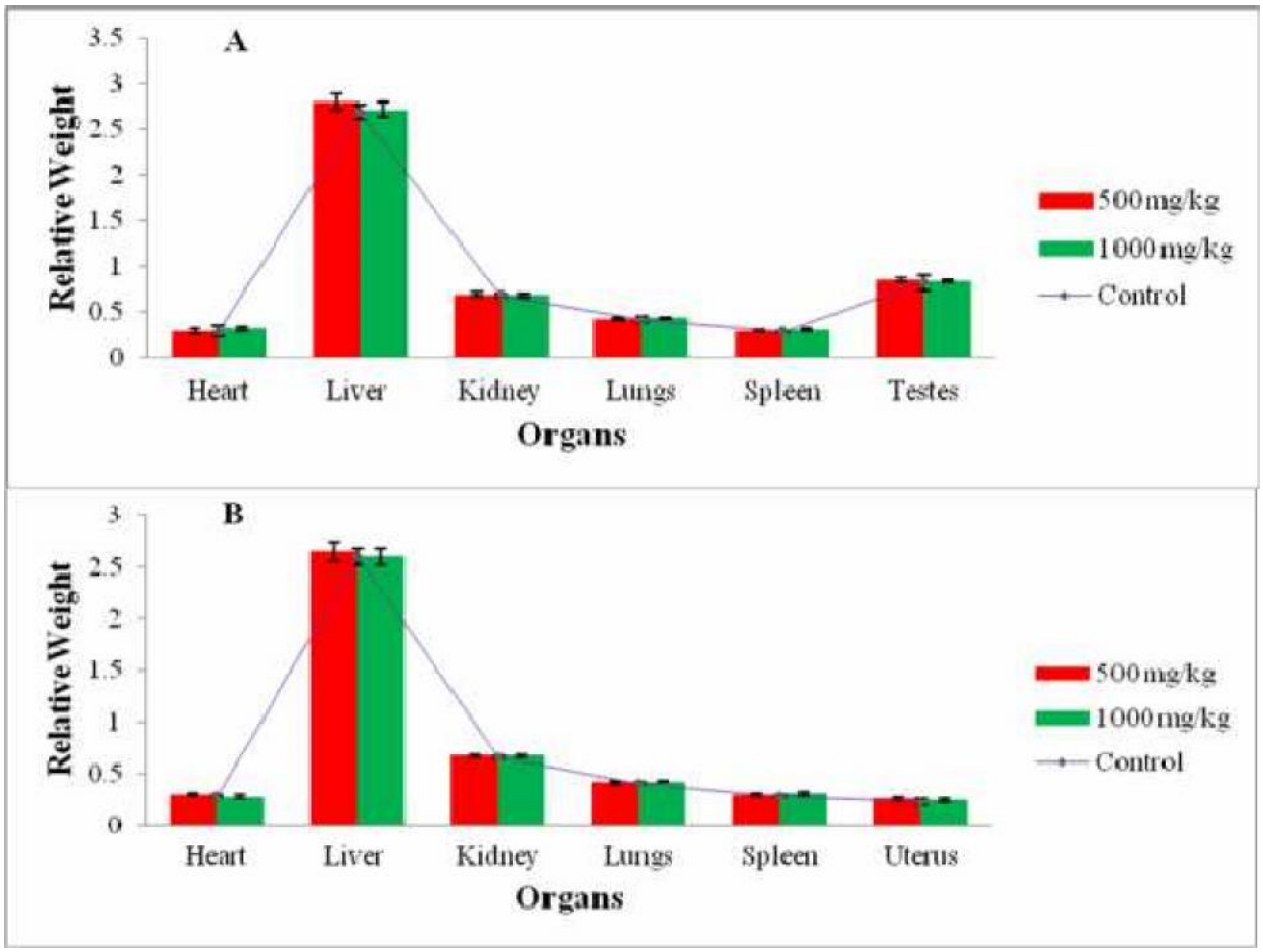

Figure 2 Relative weight of different organs of rats on 29th day after treated orally with control, aqueous extract of bark of *Ficus benghalensis* (AFB 500 & 1000 mg/kg) for 28 days. Results are mean ± SEM. ANOVA, $p > 0.05$ between groups for the same organ. **A** for male rats and **B** for female rats.

Passive avoidance test

In this, mice treated with the AFB 150 for 7 days increases the step down latency in comparison to mice treated with vehicle ($p<0.05$). Vit C also increases the SDL in

comparison to vehicle treated mice and are statistically significant (Figure 4) ($p<0.01$).

Table 4: Biochemical parameters of male rats treated with aqueous extract from the bark of *F. bengalensis* at the doses of 500 & 1000 mg/kg/day in the sub chronic oral toxicity study.

Parameters	Control	500mg/kg	1000mg/kg
ALT (U/L)	32.23 ± 2.32	31.53 ± 4.21	30.45 ± 3.43
AST (U/L)	124 ± 4.21	123 ± 4.08	121 ± 4.10
ALP (U/L)	157 ± 3.24	153 ± 3.27	152 ± 3.25
T. Protein (g/dl)	5.24 ± 0.12	5.42 ± 0.19	6.08 ± 0.14*
Albumin (g/dl)	3.62 ± 0.05	3.74 ± 0.07	3.82 ± 0.08
B. direct (mg/dl)	0.08 ± 0.02	0.09 ± 0.03	0.13 ± 0.04*
B. total (mg/dl)	0.21 ± 0.03	0.35 ±0.04*	0.39 ± 0.11*

All values are expressed as mean ± S.E.M., n=5. ANOVA followed by dunnet's test. * $p<0.05$ when the values in the groups are statistically significant against the control value.

Table 5: Biochemical parameters of female rats treated with aqueous extract from the bark of *F. bengalensis* at the doses of 500 & 1000 mg/kg/day in the sub chronic oral toxicity study.

Parameters	Control	500mg/kg	1000mg/kg
ALT (U/L)	28.32 ± 2.23	28.21 ± 3.19	28.19 ± 2.53
AST (U/L)	116 ± 2.34	113 ± 4.08	109 ± 3.23
ALP (U/L)	130 ± 3.22	126 ± 3.21	125 ± 4.24
T. Protein (g/dl)	5.42 ± 0.16	5.58± 0.42	6.91 ± 0.21
Albumin (g/dl)	2.65 ± 0.04	2.79± 0.06	2.84± 0.05*
B. direct (mg/dl)	0.06± 0.05	0.18± 0.06	0.28± 0.29*
B. total (mg/dl)	0.18± 0.03	0.31± 0.19*	0.39± 0.28*

All values are expressed as mean±S.E.M., n=5. ANOVA followed by dunnet's test. * $p<0.05$ when the values in the groups are statistically significant against the control value.

Locomotor activity test: Possibility of interference of general sensorimotor function, motor behavior during cognitive tasks, in mice were assessed by an actophotometer and they are not statistically significant and the results are shown in the table 6.

DISCUSSION

In this study, memory was assessed using the plus maze and the step-down avoidance test. The effect AFB on memory impairment induced by scopolamine in young mice and old age mice were studied. Scopolamine interferes with memory and cognitive function in humans and experimental animals by blocking muscarinic receptors *(Kopelman and Corn, 1988)*. This experimental animal model of scopolamine- induced amnesia has been extensively used in research to screen for drugs with potential therapeutic value in dementia *(Mishima et al., 2003 and Rubaj et al., 2003)*. Here, young mice treated with AFB at two doses level showed decresed transfer latency and increased step down latency than mice from control group. Also, the old age mice, treated 14 days with AFB decreased TL and increased SDL to their control group and is comparable to vit C treated mice.

Ficus bengalensis is a plant used worldwide in traditional medicine for the treatment of various ailments (*The wealth of India 2005*). The present work evaluates the acute and sub chronic toxicity of the aqueous extract of its stem bark. The results demonstrate a lack of toxicity following oral administration of AFB at a dose as high as 5000 mg/kg in the acute toxicity study. In the acute toxicity study, AFB caused neither treatment-related signs of toxicity nor mortality during 14 days of the study. Therefore, it is safe to state that its oral LD_{50} is greater than 5000 mg/kg. This resulted in classifying the extract as unclassified in the acute toxicity hazard categories according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) *(OECD, 2001)*. The substances that present LD_{50} higher than 5000mg/kg by oral route can be considered practically non-toxic.

Table 6 Effect of different doses of AFB and vit C on locomotor activity of scopolamine induce amnesia in young Swiss albino mice and old Swiss albino mice.

Treatments	Dose	Score of young mice	Score of old mice
Control	-	213.75 ± 4.80	210.63 ± 5.30
AFB 150	150mg/kg	198.50 ± 8.43	206.75 ± 8.67
AFB 300	300mg/kg	192.43 ± 7.32	195.54 ± 7.13
Vit C	250mg/kg	207.75 ± 4.05	205.25 ± 4.85

Data are expressed as means±S.E.M. (n=8). Not significant for AFB (150, 300mg/kg) and Vit-C (250mg/kg) versus control group in both young and old age mice.

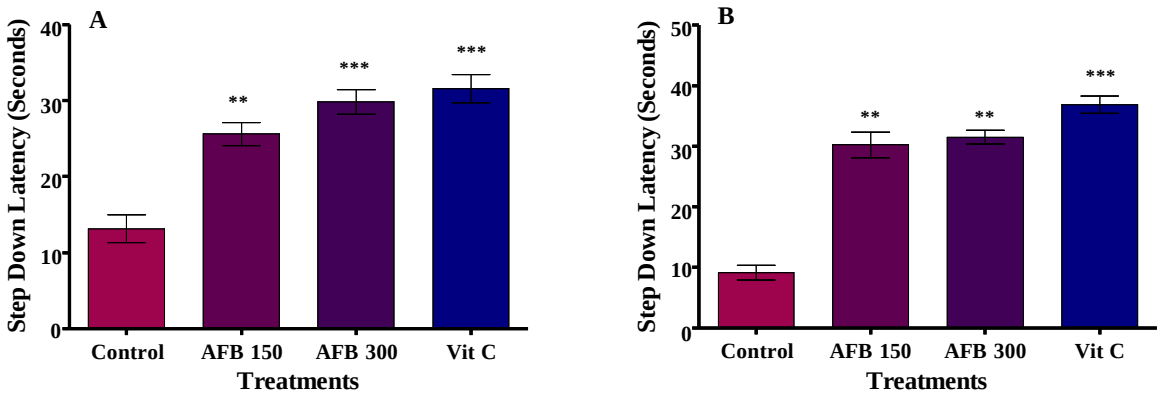

Figure 3 Effect of administration of Control, AFB (150, 300mg/kg) and Vit C (250mg/kg) for 7 days on the plus maze test. **A**: amnesia was induced by scopolamine after the 60minutes of administration of last doses on 7th day of the experiment to the young mice. Acquisition trials were carried out 30 min after scopolamine treatment. Retention trials were carried out 24 h after the acquisition trials (8th day of the experiment). **B**: Acquisition trials were carried out 60 min after last doses of the treatment to the old age mice. Retention trials were carried out 24 h after the acquisition trials (8th day of the experiment). Data are expressed as means ±S.E.M. (n = 8). **p < 0.01 and ***p < 0.001, versus control group in both **A** and **B**.

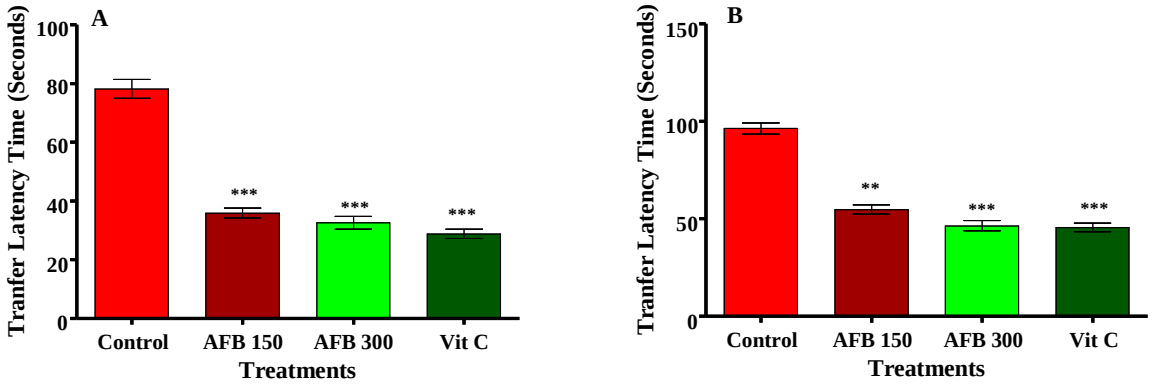

Figure 4 Effect of administration of Control, AFB (150, 300mg/kg) and Vit. C (250mg/kg) for 7 days on the passive avoidence test. **A**: amnesia was induced by scopolamine after the 60minutes of administration of last doses on 7th day of the experiment to the young mice. Acquisition trials were carried out 30 min after scopolamine treatment. Retention trials were carried out 24 h after the acquisition trials (8th day of the experiment). **B**: Acquisition trials were carried out 60 min after last doses of the treatment to the old age mice. Retention trials were carried out 24 h after the acquisition trials (8th day of the experiment). Data are expressed as means ±S.E.M. (n = 8). **p < 0.01 and ***p < 0.001, versus control group in both **A** and **B**.

Therefore, it can be suggested that AFB is devoid of acute oral toxicity. *(Kennedy et al. 1986)*. In the repeated dose 28-days oral toxicity study, no deaths and no treatment-related signs were observed in animals of all groups. Although the body weight of female rats treated with the AFB at both the dose (i.e.500mg/kg & 1000mg/kg) is slightly but significantly increased that of its control counterpart on day 28 of the study, however, weight changes of in all groups showed no significant difference at the end of each week. In addition, there, slight decrease in weight was observed in male rats and in group treated with 1000mg/kg/day. These results may be due to biological variation with a lack of biological significance. The absolute organ weights in all treated groups of both sexes at the doses level of 500 &1000 mg/kg/day in the repeated dose 28-day oral

toxicity study were not significantly different from their respective control groups with the exception of the liver weight of male & female rats was slightly lower than the controls at dose level 1000mg/kg/day. The slight changes in the absolute and relative organ weights were considered not to be treatment related because the values were within the normal laboratory range and no abnormality was noted with respect to gross or histopathological examinations of all organs examined. Analysis of blood parameters is relevant to risk evaluation as the changes in the haematological system have a higher predictive value for human toxicity, when the data are translated from animal studies *(Olson et al., 2000)*. The haematological profile of treated rats showed no significant difference with control group, except WBC which decreases in the group treated with AFB at the dose 1000 mg/kg in both male and female rats. Although not significant, a global increase was observed in red blood cell count, haematocrit, haemoglobin concentration, implying that there may be a possible increase in erythropoiesis with increasing doses of AFB. In the present study, a significant rise in total and conjugated bilirubin was also observed and it is obviously known that increase in bilirubin levels suggests increase in haemolysis *(Orish et al., 2003)*. Thus, the increase in bilirubin level may indicate an elevated level of haemolysis. This may be easily understood and not indicating any toxic effect. Since there is increase in total number of red blood cells, the number of red blood cells that will undergo haemolysis will increase given that the lifetime of these cells is very short. The increase levels of AST and ALT in the blood are associated with damage of hepatic cells *(Witthawaskul et al., 2003; Burger et al., 2005)*. Also, *Emerson et al. (1993)* have reported that enhancement in the level of serum proteins is an indication of tissue injury and reflection of hepatic toxicity. Sub-chronic administration of AFB caused significant decrease in the levels of total protein, AST, ALT and ALP. These observations of significant decrease in the levels of liver enzymes may indicate that AFB has hepatoprotective effects.

CONCLUSIONS

On the basis of results of passive avoidance test and the plus maze tests on scopolamine induced amnesia in young mice and amnesia due to age in old mice, we found that AFB had cognitive-enhancing activity. AFB that exerts anti-amnesic activity in *in vivo* might offer a useful therapeutic choice in the treatment of Alzheimer's disease. The high single dose administration of AFB (5,000 mg/kg) of body weight did not show acute oral toxicity in rats in present study. In the sub-chronic 28 days oral toxicity test, almost no obvious toxic changes which due to administration AFB were observed in any parameters. Therefore, the No Observable Adverse Effect Level (NOAEL) in this sub chronic toxicity study was 1000 mg/kg/day for both sexes in rats. The present study suggested that high dose (1000 mg/kg/day) of aqueous extract from the leaves of *Ficus bengalensis* intake would be well-tolerated for long-term use as a dietary supplement whatever by animals or human.

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