Morus alba; A Ray of Hope for Psychiatric Illnesses

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Abstract

Purpose: Over the years, plants have been used in treatment of various health ailments. Morus alba also known as White Mulberry is a deciduous tree which belongs to family Moraceae. Mulberry is rich in antioxidants such as hydrophobic flavonoids, polyphenols, carotenoids, vitamin A, C and E.

Materials and Methods: This study analyses the central nervous system activities including memory enhancement, learning abilities, changes in behavior, cognition, anxiety and depression. These effects will be studied on experimental mice, which will be distributed in control and test groups. Aqueous fruit extract prepared by Maceration extraction will be given to test group in three different doses per oral over a time period of 30 days, whereas control will be given water for injection for same duration.

Findings: Afterwards, changes in aforementioned effects will be observed to determine the pharmacological effects of taken fruit. The obtained results suggest an antidepressant like effect of mulberry in the initial two weeks of dosing. Afterwards it shows anxiolytic effect at higher doses.

Implications to Theory, Practice and Policy: All these tests can be repeated weekly while dosing the animals daily for 90 days for chronic toxicity analysis. Furthermore, we can add a brain biopsy/histopathology to evaluate the changes in chemical neurotransmitters.

Keywords: Morus alba, White Mulberry, Anxiolytic, Anxiogenic, Antidepressant, Antioxidant, Maceration
1.0 INTRODUCTION

Traditional medicine is known to have reliance on phytochemicals enriched plant extracts to cure different type of ailments. Genus *Morus* (Mulberry) is one such example having over 150 species, among which *Morus alba* is dominant (Dkhil, M. A., Bauomy, A. A., Diab, M. S., & Al-Quraishy, S., 2015). The Pharmacological effects of *Morus alba* will be studied on animal model, which will be distributed in control and test groups. Fruit of this plant will be given to test group over a time period of 30 days, whereas control will be given water for injection for same time. Afterwards, improvement in Cognition, learning ability, Behavior, Anxiolytic and Antidepressant effects will be studied to determine the pharmacological effects of plant.

**Background**

*Morus alba* is a deciduous tree which belongs to family *Moraceae* (Vijayan, K., Saratchandra, B., & da Silva, J. A. T., 2011). The genus *Morus* is distributed all over Asia, Europe, North America, South America and Africa. This wide pattern of its distribution reveals the fact that it can grow in a variety of climatic, topographical and soil types. Mulberry trees can grow from sea level to altitude as high as 4000 meters. They are grown in the Himalaya-Hindu Kush region, whereas in Pakistan, it is cultivated in Northern regions of Gilgit Baltistan including Hunza, Skardu, Astore and Gilgit city (Dkhil, M. A., Bauomy, A. A., Diab, M. S., & Al-Quraishy, S. 2015).

**Chemical Composition**

*Morus alba* has high quantities of Flavones, triterpenes, proteins, amino acids, carbohydrates, fats, fibers, minerals and vitamins such as vitamin C and its precursors. In a study it has been stated that *M. alba* leaves contain high contents of crude protein (25%) (De Souza, V. R., Pereira, P. A. P., Da Silva, T. L. T., de Oliveira Lima, L. C., Pio, R., & Queiroz, F., 2014). Mulberry fruit consists of Moranolin, Moran (glycopeptides) and hydrophobic flavonoids which are responsible for the hypoglycemic effect. It has been reported that mulberry contains active compounds that act as antioxidant like polyphenols, carotenoids and vitamin A, C and E (Yang, Z. G Kitanaka, S. 2011).

These constituents contribute in antioxidant status of the body and controls low density lipoprotein (LDL) oxidation via various mechanisms. Mulberry fruit has cyaniding-3-O-β-glucopyranoside that hinders neuronal cell damage. In a study many chemical compounds were identified such as kuwanon S, ciclomulberrin, ciclomorusin, morusin, 8 granilapigenin, atalantoflavones, kaempherol having strong effect on cancer cell lines for example HeLaMCF-7 and Hep3B (Kim, S. B., Chang, B. Y., Jo, Y. H., Lee, S. H., Han, S. B., Hwang, B. Y., ... & Lee, M. K., 2013), (Andallu, B., Suryakantham, V., Srikanthi, B. L., & Reddy, G. K., 2001).

*Morus alba* has highest total fat content (1.10%). The major fatty acids in mulberry fruits are linoleic acid (54.2%), Palmitic acid (19.8%) and oleic acid (8.41%) (Ercisli, S., & Orhan, E. 2007). The Mulberry fruits are rich in total phenol and alkaloid contents, having values: 880-1650mg/100g FW and 390-660mg/100g FW, respectively (Imran, M., Khan, H., Shah, M., Khan, R., & Khan, F. 2010).

In a study, bioactivity-guided fractionation of *Morus alba* fruit extract resulted in isolation and separation of 25 phenolic compounds for the first time. Compounds 1-8 were flavonoids with sugar moieties including Quercetin glucopyranoside, quercitin rutinoside, kaempherol. Compounds 12-14 were chalcones and derivatives including isobavachalcone, morachalcone. Compounds 9-11
were derivatives of Flavonones such as dihydrokaempferol. Compounds 15-55 were Phenolic acid derivatives such as jaboticabin, protocatechuic acid, vanillic acid (Wang, Y., Xiang, L., Wang, C., Tang, C., & He, X, 2013).

In another study, for the first time these compounds were isolated from cell cultures of *Morus alba* using column chromatography on silica gel and semi-preparative High performance liquid chromatography technique. Eight compounds were specifically separated and purified (Tao, X. Y., Zhang, D. W., Chen, R. D., Yin, Y. Z., Zou, J. H., Xie, D., ... & Dai, J. G, 2012) which are as follows:

1. Isobavachalcone
2. Genistein
3. Norartocarpetin
4. Albanin A
5. Guangsangon
6. Mulberrofuran F
7. Chalcomoracin
8. Kuwanon

![Figure 1: Mineral Content of Morus alba](Tao, X. Y., Zhang, D. W., Chen, R. D., Yin, Y. Z., Zou, J. H., Xie, D., ... & Dai, J. G 2012)

**Health Impact**

Epidemiological and experimental findings show a negative relation between the intake of fruits and vegetables versus the risk for growing chronic angiogenic diseases such as neurodegenerative
diseases such as Parkinson, Alzheimer. It has been studied that fruit of *Morus alba* has high medicinal and unique nutritional value. *Morus alba* is also used in traditional medicine system, claimed to have a beneficial effect on various body systems and organs including neuro-protective effects (Iqbal, M., Mir, K., & Munir, M. 2010), (Zafar, M. S., Muhammad, F., Javed, I., Akhtar, M., Khaliq, T., Aslam, B., ... & Zafar, H. 2013).

Every part of this plant is of great medicinal value and their mechanism of action is dependent on anti-oxidative action. The tea leaves of *Morus alba* is known to possess antidepressant effects. Various studies verifies its Neuro-protective, Cognition enhancing effect and beneficial for overall well-being (Andallu, B., Suryakantham, V., Srikanthi, B. L., & Reddy, G. K. 2001), (Yao, C. H. E. N., Qian, L. I., Ye, Z. O. U., Xiang, Z. Z., Wei, F. W., Tuan, B. Y., ... & Yang, 2014), (Lee, J. 2011). It was found that cyanidin-3-O-β-D-glucopyranoside (C3G) from *Morus alba* fruit extract contributes in the neuroprotective effect on neuronal cell damage induced by hydrogen peroxide causing oxygen glucose deprivation (OGD). The fruit extract inhibited cerebral ischemic damage (Kang, T. H., Hur, J. Y., Kim, H. B., Ryu, J. H., & Kim, S. Y. 2006).

*Morus alba* fruit extract improved oxidative stress and enhanced densities of neuron and cholinergic neuron in hippocampus. Also, enhanced density of Bcl-2-immunopositive neurons and suppression of Acetylcholinesterase were also observed with increased spatial memory in animal model of vascular dementia (Kaewkaen, P., Tong-un, T., Wattanathorn, J., Muchimapura, S., Kaewrueng, W., & Wongcharoenwanakit, S. 2012).

50.2mg of elemental Zinc was found in *Morus alba* fruit in a study. The effect of Zinc with supplementation of vitamin A improved long term memory as well as resulted in high nerve growth factor in lab mice in a three month study. The NGF (Nerve growth factor) was observed in hippocampus, cerebellum and cerebral cortex. And memory was verified by radial arm maze (Kheirvari, S., Uezu, K., Sakai, T., Nakamori, M., Alizadeh, M., Sarukura, N., & Yamamoto, S 2006).

Role of Zinc in neurological conditions such as stroke, epilepsy & alzheimer’s disease has been studied. It has been demonstrated in a study that Zinc ion alters conformation of NGF, making it unable to bind to p75 or TrkA receptors or to activate signal transduction pathways & the biological outcomes normally induced by this protein (Ross, G. M., Shamovsky, I. L., Lawrance, G., Solc, M., Dostaler, S. M., Jimmo, S. L., ... & Riopelle, R. J. 1997).

Mulberry fruit consists of vitamin A & E as reported in a study. Vitamin E when compared to Piracetam which is a nootropic agent, caused increase in neuronal cell size and volume in lab rats in CA1 pyramidal layer of hippocampus, also showed a spontaneous improvement in behavior in the Y-maze. These findings suggest neuro-protective effect of Vitamin E. In a clinical study, high dose of vitamin C and E given in patients with acute craniocerebral injury turned out to decrease nerve injury, oxidative stress response and improve neurotrophic state. Blood was collected and serum was isolated on day 3 and 7 after treatment to analyse the nerve injury index and neurotrophic indices (Zhang, C., Li, J. M., Hu, J. L., & Zhou, X 2018).

It was found that administration of vitamin A has same therapeutic effect as ATRA (all-trans retinoic acid) on peripheral neuropathy and suggest a potential therapeutic use in patients with diabetes. ATRA is known to promote endogenous expression of both NGF and retinoic acid beta receptor beta (RAR- β). In a study on 70 rats divided in 4 groups where vitamin A was used in
dose of 20,000 IU. Treatment with vitamin A reverted the sensorial disturbances due to streptozotocin induced neuropathy (Hernández-Pedro, N., Granados-Soto, V., Ordoñez, G., Pineda, B., Rangel-López, E., Salazar-Ramiro, A., ... & Sotelo, J., 2014).

15.20mg fresh weight of Ascorbic acid was isolated from Mulberry fruit. The bioeffects of Ascorbic acid can improve the morphological and functional recovery of degenerated peripheral nerves. The main targets of effects are neurons, Schwann cells and macrophages. This was studied on lab mice with sciatic nerve press injury where it was observed that Ascorbic acid can speed up axonal regrowth in early days post-injury. On 28 days post injury it increased density, size and re-myelination of axons in injured nerves (Guo, J., Li, L., Li, Y., Fan, Z., Wang, X., Li, Z., ... & Hu, X 2019).

In another study using High performance liquid chromatography Genistein was separated and purified from *Morus alba* fruit. Flavonoids are known for their protection in brain diseases, which was confirmed in a study on cultured rat astrocytes was performed for illustrating effects of flavonoids on regulating neurotrophic factors. Astrocytes are cells in brain that secrete neurotrophic factors. Genistein was one of those 33 flavonoids that were screened and found to be highly active in inducing synthesis and secretion of neurotrophic factors including: GDNF (Glial derived neurotrophic factor), NGF, BDNF (brain derived neurotrophic factor). Therefore, such supplements should be taken by patients suffering from neurodegenerative diseases (Xu, S. L., Bi, C. W., Choi, R. C., Zhu, K. Y., Miernisha, A., Dong, T. T., & Tsim, K. W., 2013).

Genistein enhances NGF induced neurite outgrowth of PC12 cells. But it was found that NKCC1 co transporter isoform 1 (Na+/K+/2Cl- ) is essential for it to stimulate the neurite outgrowth. Therefore, genistein enhanced the NGF-induced neurite outgrowth in PC12 cells via activation of NKCC1 (Nakajima, K. I., Niisato, N., & Marunaka, Y., 2011).

Almost 0.088mg Fresh weight was identified of Niacin in mulberry fruit. Niacin (nicotinic acid) is highly effective in clinical use for increasing HDL-C (high density lipoprotein cholesterol). In a study it was hypothesized that niacin treatment of stroke promotes synaptic plasticity and axon growth in ischemic brain in rats. Whereas HDL-C is highly important in maintaining homeostasis of cell membrane cholesterol and has a role in regulation of synaptic function and cell plasticity (Cui, X., Chopp, M., Zacharek, A., Roberts, C., Buller, B., Ion, M., & Chen, J., 2010).

Neither any signs of toxicity nor death of rats has been found in a 14 day trial with dose 2000mg/kg given per orally to rats (Kaewkaen, P., Tong-un, T., Wattanathorn, J., Muchimapura, S., Kaewrueng, W., & Wongcharoenwanakit, S., 2012), (Yadav, A. V., & Nade, V. S. 2008) and in a 90 day study conducted by Chang, Kin, Lee, Park & Kim in 2016 (Chang, B. Y., Kim, S. B., Lee,
M. K., Park, H., & Kim, S. Y., 2016), where dose given is upto 1000mg/kg per orally. The results also suggest that Mulberry does not have any genotoxicity potential.

**Mulberry as Antipsychotic**

The leaf is used traditionally for psychiatric conditions. In an animal trial referred in immortal musings in 2014, 100mg/kg mulberry leaf extract was found equally effective to clozapine in reducing psychosis (head twitches) which is an antipsychotic agent used for severe schizophrenia (Kenney R. 2014).

Yadav & Nade in 2008 performed a series of mental tests by various inducers effect of methanolic extract of *Morus alba* was studied. Inducers including; catalepsy by haloperidol, metoclopramide, foot-shock induced aggression, amphetamine-induced stereotyped behavior and phenobarbitone induced sleeping in mice. It was concluded that the extract possesses anti-dopaminergic activity. The results included; reduced number of fights, increased latency to fights, decreased amphetamine induced stereotyped behavior in dose dependent manner, sleeping time was prolonged in phenobarbitone tests, inhibited contractions produced by dopamine on isolated rat vas deferens.

**2.0 MATERIALS AND METHODS**

The tests were performed to screen improvement in cognition, learning ability and changes in behavior as well as to evaluate anxiolytic and antidepressant activities. This study was performed in Pharmacology Lab after approval from the Ethical Committee of the Department of Pharmacology in Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Pakistan.

Fresh *Morus alba* fruit were identified and collected from Hunza Valley, Northern region of Pakistan. The sample was sun dried for 48 hours and packed in polythene bags for travel to Karachi by air route within 7 days. The Mulberry fruit extract was prepared by maceration extraction using distilled water as medium. A measured quantity of crude drug is comminuted using a mechanical grinder. Then it is soaked in a measured quantity of solvent for 10 hours until the cellular structure is softened and penetrated by solvent and soluble constituents are dissolved and extracted. A stock solution of 0.8g/ml was prepared. Five groups of ten experimental albino mice (mix genders) were given control (Water for injection), standard (given below) and three different test doses of mulberry fruit per oral for 30 days as follows:

**Table 1: Dosing in Mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Average Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.1ml WFI</td>
<td>27g</td>
</tr>
<tr>
<td>Standard</td>
<td>Variable*</td>
<td>26g</td>
</tr>
<tr>
<td>Test 1</td>
<td>7mg/kg</td>
<td>23g</td>
</tr>
<tr>
<td>Test 2</td>
<td>12mg/kg</td>
<td>27g</td>
</tr>
<tr>
<td>Test 3</td>
<td>16mg/kg</td>
<td>29g</td>
</tr>
</tbody>
</table>

*Diazepam and Fluoxetine were administered at dose 1mg/kg depending on method mentioned*
All the mice were given standard animal feed and water, room temperature maintained at 24ºc (+-2) with 45-55% humidity in the facility. They were housed in groups of 5 in animal cages with 12 hour dark and light cycle.

**Open Field Test**

In this test, an open arena comprising of a see-through floor space of 40 by 4 cm and 30 cm high walls. The floor is divided in nine equal square boxes by black lines. Mouse is placed in middle of the field and left un-disturbed for few minutes to get acquainted to the open field environment. Afterwards, the following parameters are recorded for the next 5-6 minutes.

a) No. of squares covered
b) Rearing (no. of times animal stands on its rear paws)
c) Grooming
d) No. of entries in center square

Open field observes behavior variations in animals exposed to new environments and to identify angiogenic and anxiolytic activities under similar conditions. It also monitors locomotor activity, impairment in neuromuscular diseases and efficacy of respective drugs (Tatem, K. S., Quinn, J. L., Phadke, A., Yu, Q., Gordish-Dressman, H., & Nagaraju, K., 2014). This test was performed on 7th, 14th, 21st & 28th day of daily dosing with extract.

**Light and Dark Transition Test**

An apparatus containing a box having a dark and a light chamber is used. Mice are placed one at a time in light chamber and allowed to roam freely within the chambers which are partitioned by an open door. The time taken in each chamber and number of entries made in each chamber is recorded and it is used to measure anxiety related performance. This test is based on the likeness of mice to brightly illuminated areas and their spontaneous exploratory activity in response to mild stressors, for example; novel environment and light. So, we can use this method to test anxiolytic drugs. One benefit of this test over others is that it doesn’t need any training of animal and is a quick and easy to use method (Takao, K., & Miyakawa, T. 2006). This test was performed on 7th, 14th & 21st day of daily dosing with extract.

**The Novel Object Recognition (NOR)**

This animal paradigm is used as an evaluation of cognition and learning particularly, memory in rodent models of central nervous conditions. It is based on the ability of animal to spend more time in learning about new object rather than a known one. Its priority to learn the novel object shows the application of recognition, memory and learning. The idea lies on the fact that rodents have innate exploratory behavior and are curious about new objects. The first time it sees an object it will investigate and spend time with it but later on having the same object presented to which it won’t show same level of interest.

This test includes the use of an open field arena with two different objects on basis of color, shape and dimension. First the mouse is made familiar with the empty arena (habituation). After twenty four hours the animal is again put into the same arena with two look-alike objects kept at an equal distance. Next day, the animal is kept in the same place now with a familiar object and a novel object to test long term recognition memory. The time spent with each object are recorded (Savage,
This test was performed only once to identify the short-term memory.

**Forced Swim Test (FST)**

This test includes a water tank in which an animal is placed from which it cannot escape. The animal tries a few attempts to escape at first then will show immobility eventually i.e. floating without moving except to keep its nares above water level. Transparent cylindrical Plexiglas water tanks are used that can withstand frequent movements and accidents. Water is kept at 15 cm from the bottom and is marked on the tank to be kept consistent. This test is used to evaluate new and existing antidepressant drugs. The principle lies on the fact that the animal is kept under stress to produce depression due to its lack of ability to deal with stress. A successful FST requires adherence to procedural details and minimizing unwarranted stress on animal. The water in the tank has to be changed after each trial (Can, A. et. al. 2012), (Yankelevitch-Yahav, R., Franko, M., Huly, A., & Doron, R., 2015). This test was performed on 7th and 15th day of daily dosing with extract.

**Observation**

No. of animals per group (n) = 10

All values are mean

M1: Test group one
M2: Test group 2
M3: Test group 3

**Table 2: Open Field Test**

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of sq.</td>
<td>Rears</td>
<td>Center sq.</td>
<td>Grooming</td>
</tr>
<tr>
<td>Control</td>
<td>150</td>
<td>7</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Diazepam (1mg/kg)</td>
<td>168</td>
<td>15</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>M1</td>
<td>167</td>
<td>13</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>M2</td>
<td>144</td>
<td>33</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>M3</td>
<td>143</td>
<td>41</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>168</td>
<td>11</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Diazepam</td>
<td>200</td>
<td>18</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>M1</td>
<td>118</td>
<td>17</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>M2</td>
<td>80</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>M3</td>
<td>87</td>
<td>30</td>
<td>1</td>
<td>4</td>
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</tbody>
</table>
Table 3: Light and Dark Transition Test

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 1</th>
<th></th>
<th></th>
<th>Week 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tl</td>
<td>Td</td>
<td>El</td>
<td>Ed</td>
<td>Tl</td>
<td>Td</td>
</tr>
<tr>
<td>Control</td>
<td>92</td>
<td>208</td>
<td>6</td>
<td>8</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>Diazepam (1mg/kg)</td>
<td>150</td>
<td>150</td>
<td>10</td>
<td>9</td>
<td>155</td>
<td>145</td>
</tr>
<tr>
<td>M1</td>
<td>140</td>
<td>160</td>
<td>5</td>
<td>5</td>
<td>290</td>
<td>10</td>
</tr>
<tr>
<td>M2</td>
<td>156</td>
<td>144</td>
<td>8</td>
<td>7</td>
<td>43</td>
<td>257</td>
</tr>
<tr>
<td>M3</td>
<td>198</td>
<td>102</td>
<td>8</td>
<td>7</td>
<td>90</td>
<td>210</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 3</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tl</td>
<td>Td</td>
<td>El</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>200</td>
<td>6</td>
</tr>
<tr>
<td>Diazepam</td>
<td>160</td>
<td>140</td>
<td>10</td>
</tr>
<tr>
<td>M1</td>
<td>300</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>M2</td>
<td>285</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>M3</td>
<td>210</td>
<td>90</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 4: Novel Object Recognition Test

<table>
<thead>
<tr>
<th></th>
<th>Time with familiar object (seconds)</th>
<th>Time with novel object (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>M2</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>M3</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 5: Forced Swim Test

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 1</th>
<th></th>
<th></th>
<th>Week 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Climbing</td>
<td>Immobility</td>
<td>Swimming</td>
<td>Climbing</td>
<td>Immobility</td>
<td>Swimming</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>275</td>
<td>10</td>
<td>10</td>
<td>290</td>
<td>0</td>
</tr>
<tr>
<td>Fluoxetine (1mg/kg)</td>
<td>20</td>
<td>270</td>
<td>5</td>
<td>30</td>
<td>220</td>
<td>50</td>
</tr>
<tr>
<td>M1</td>
<td>70</td>
<td>215</td>
<td>15</td>
<td>192</td>
<td>135</td>
<td>20</td>
</tr>
<tr>
<td>M2</td>
<td>60</td>
<td>40</td>
<td>200</td>
<td>0</td>
<td>245</td>
<td>115</td>
</tr>
<tr>
<td>M3</td>
<td>80</td>
<td>150</td>
<td>70</td>
<td>20</td>
<td>230</td>
<td>96</td>
</tr>
</tbody>
</table>

3.0 FINDINGS & DISCUSSION

Open Field Activity

In control group locomotor activity increases in each passing week. Rearing also increases with each passing dose which shows attempt to escape and exploratory behavior. Anxiolytic/risk taking behavior is normally in increasing trend. Stereotypi is recognized by grooming which also increases. Stereotypi is a time spending behavior in animals. In Standard group (Diazepam),
exploratory & locomotor activity is found to be in increasing trend. Similarly increased trend is observed in rearing. No of entries in center square also increases with each passing week. Stereotypi is observed to increase with each passing week.

In test group one locomotor activity increases on 7th and 14th day of dosing, which gradually decreases on 21st & 28th day of dosing. This could be suggestive of decreased dose response over time. This is confirmed by no of rearing which increases on 7th & 14th day. It gradually decreases on 21st & 28th day of dosing. This could mean that initially dose responds well giving positive exploratory behavior but decreases over time. A decrease trend is observed in the no. of entries in center square which shows anxiogenic effect. The stereotypi increases in first two weeks and decreases gradually over next two weeks.

In test group two, no of squares traversed is increased on 7th day of dosing, which decreases gradually on 14th, 21st & 28th day of dosing. Rearing activity also shows a decreasing trend over the course of dosing. Entries made in center square also decreases over time. Whereas stereotypi behavior fluctuates in between decreasing & increasing trend. All this shows increased locomotor & exploratory activity in initial dosing period which gradually decreases later on. This could be suggestive of dose dependent anxiolytic effect.

In test group three, no of entries in center square decreases with time, stereotypical grooming decrease on 7th day and increases gradually over 14th, 21st & 28th day of dosing. No of rears made on 7th day is increased which decreases gradually through each passing week. No. of peripheral squares crossed is in increasing trend but it decreases abruptly on 21st day of dosing. All this suggests a mix of exploratory & locomotor effects found at this highest dose.

**Light and dark paradigm test;** mice tend to avoid lit areas and has natural inclination to stay in dark.

In control group, time spent in light area was less than in dark on 7th, 14th and 21st day of dosing. The exploratory behavior is slightly less than standard throughout the dosing period shown by less number of entries made in both areas.

In diazepam treated group, on 7th day of dosing, spend equal amount of time in both areas. On 14th & 21st day of dosing, mice spend slightly more time in light area than in dark showing the anxiolytic effect of drug. Increased number of entries in both the areas shows good exploratory behavior.

In test group one, time spent in light area increases over the course of treatment, and decreased time spent in dark area was observed. This explains their exploratory and anxiolytic behavior. The number of entries in both areas were increased in 7th and 14th day of dosing and decreased drastically in 21st day of dosing which clearly indicates anxiolytic behavior.

In test group two, time spent in both areas were approximately same, at 14th day time in light area decreased significantly and in dark time spent increased. On 21st day of dosing time spent in light area reached maximum and time in dark decreased to minimum. This effect can be due to increase in dose. Initially mice shows exploratory behavior, then at 14th day shows anxiogenic effect. At 21st day shows anxiolytic behavior. No of entries in both areas is greater as compared to test group one. This also directs toward more exploratory behavior in mice at this dose.
In test group three, time spent in light area is greater than time spent in dark area on day 7th of dosing. Then on 14th day, time spent in light area decreases significantly as compared to time spent in dark area, showing anxiogenic effect. On 21st day of dosing time spent in light area increases showing anxiolytic effect. This is also an indication of dose dependent effect.

Increased no. of entries in both areas confirm exploratory behavior in mice.

In the **Novel Object Recognition test** we are observing tendency of mice to explore novel object, its recognition & memory.

In test group one; mice spend less amount of time with the old (same) object that is repeated twice. On introducing a new object, mice spends more time with it showing increased learning & exploratory behavior. Also they spend almost equal amount of time recognizing the old object (time spend with old object day before and day after novel object) showing that it got good short term memory.

In test group two; mice shows good exploratory and learning ability upon introducing novel object. Here they spend lesser time with old object on day after as compared to day before which shows quick recognition & excellent short term memory.

In test group three; mice shows good exploratory and learning behavior with novel object by spending more time with it. It also suggests good short term memory.

**Forced swim test** induces depression as it bounds the animal to a deep water filled tank (moderate stress). It was observed that initially (control) mice swam and climbed (attempts to escape from tank) for lesser duration due to becoming helpless or depressed and therefore, floating/immobility (remaining buoyant on surface of water) time increased. In standard group, treated with fluoxetine (SSRI antidepressant), floating time slightly decreases whereas swimming time and climbing time increases slightly. In test group one, floating time is increased significantly on 7th day of dosing then decreases on 14th day whereas, swimming & climbing time increases on 14th day of dosing. In test group two, floating time is decreased on 7th day which increases significantly on 14th day whereas, swimming and climbing time is increased on 7th day which decreases on 14th day of dosing. In test group three, floating time is increased on 7th day which increases further on 14th day whereas, swimming time is decreased on 7th day which decreases further on 14th day. Climbing time is also decreased which decreases further on 14th day.

In test group one antidepressant effect which is better than fluoxetine is observed over the 14 days period of dosing.

In test group two on 7th day of dosing swimming & climbing time are increased and floating time is decreased (antidepressant effect better than fluoxetine). On 14th day swimming & climbing time decreased, whereas floating time increased. This can be due to dose dependent anxiolytic effect.

In test group three, swimming & climbing time is decreased whereas floating time is increased on 7th day (anxiolytic effect). On 14th day of dosing, swimming time increased slightly, climbing decreased and floating time increased. This anxiolytic effect can be due to increase in dose given.

Anxiety has affected one eighth of the global population and has become an important area of research in psychopharmacology. Benzodiazepines are most often prescribed as first line for treatment of anxiety, and they are reported to have very narrow safety margin. Therefore research on other safe options with fewer side effects is still going on.
According to a study, extract of the leaves of *Morus alba* possess anxiolytic and muscle relaxant activities, which is mediated through GABAa-BZD mechanism. It can be used in treatment of anxiety and muscle tension disorders (Yadav, A. V., & Nade, V. S. 2008).

On the other hand in another study, *Morus alba* leaves became known for their antidepressant effect, however at higher doses it shows sedative effect and alters muscle strength and pain response (Sattayasai, J., Tiamkao, S., & Puapairoj, P., 2008).

### 4.0 CONCLUSION AND RECOMMENDATIONS

**Conclusion**

*Morus alba* is a versatile plant which is very beneficial specially its fruit with least number of side effects. In this study investigations were made on its enhanced memory and cognition with improved motor skills. It possesses anxiolytic as well as antidepressant effects. The fruit being tasteful and healthy can be given at clinical level and evaluated for outcomes. It is used on daily basis in the Northern areas of Pakistan and many other regions of the world contributing to longevity of healthy lives in people. The use of this fruit should be encouraged among children as a confectionary item instead of other harmful items, as it is completely organic with no side effects.

Mulberry is a healthy dessert that is used on daily basis possessing beneficial outcomes on overall health, it can be used to treat various ailments and diseases as well, as it is rich in phenols and flavonoids giving the antioxidant capacity.

**Recommendations**

All these tests can be repeated weekly while dosing the animals daily for 90 days for chronic toxicity analysis. Furthermore, we can add a brain biopsy/histopathology to evaluate the changes occurring in chemical neurotransmitters.
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