Anti-Anxiety activity of Kaahu (Lactuca sativa Linn.) and Nilofer (Nymphaea Alba Linn.) in animal model

Dr. Manumula Rajaram, Dr. Ghulamuddin Sofi, Dr. Manumula Komala Dr. Manchala Ashok and Dr. Makula Swathi

Abstract
Anxiety disorders are among the foremost common mental diseases, with immense attendant quality of life and monetary price. Unani medicine, as is well known, based on the Hippocratic humoral theory. In Unani medicine, psychiatric disorders are dealt thoroughly below the heading of “amraz-e-nafsaniya”. The term “izterab” is employed for anxiety in Arabic and Unani texts and therefore the word nafsani (Psychic) is added to izterab to specify its condition. The present study was undertaken in the department of Ilmul Advia, National Institute of Unani Medicine, Bengaluru. Wister albino rats of either sex, weighing between 150 – 200 gm were used in the study. Elevated plus-maze (EPM) test is the model used in this study to define the anxiolytic action, as this EPM test used in almost 80% of the scientific publications. Diazepam syrup was used in this study. It was given at a dose of 0.11 mg/kg/bw orally. Single unani drugs Kaahu (Lactuca sativa Linn. seeds) and Nilofer (Nymphaea alba Linn.) flowers were used as test drugs in the form of hydro alcoholic extracts. The hydro alcoholic extracts of Kaahu (Lactuca sativa Linn) at the dose of 51 mg/ kg body weight and Nilofer (Nymphaea alba Linn) at the dose 206 mg/ kg body weight were tested in the Elevated Plus Maze (EPM) for their anti-anxiety effect and observed a significant effect compared to diazepam.

Keywords: Anxiety; Unani medicine; Izterab Nafsani; Kaahu, Nilofer

Introduction
Mental problems acquire an eminent position in Unani medicine as their description has been made since the time of Greek physician, Hippocrates (460 – 377 BC) who propounded that mental diseases were the consequences of qualitative/quantitative imbalance of Akhlat [1]. In Unani system of Medicine, interpretation of mental issue depend on the teaching of Galen (d.200ca.) and Avicenna (Ibn Sina, d. 1037), and the physiology of four natural liquids called akhlaat (humors) i.e., blood, mucus, yellow and dark bile, specifically sauda (dark bile) will instigate burdensome scatters, overabundance of safra (yellow bile) results to ekhtinaqar raham (delirium) and maniacal disorders [2]. In Unani medicine, psychiatric scatters are managed altogether beneath the heading of “amraz-e-nafsaniya” wherever physicians and students sketched out fluctuated side effects of psychic faculty and their distortion because of the involvement of humors particularly “safra” and “sauda”. As per World Health Report, roughly 450 million individuals experience the ill effects of one or other mental (or) behavioural disorder [1]. This amount is around 12.3% of the global burden of disease, predicted to ascend to 15% by 2020 [2]. Anxiety disorders are the foremost common form of psychiatric disorders, with associate incidence of 18.1% in total world population and a life time prevalence of 28.8% [3].

The description of Anxiety disorders, as such, is not found in Unani literature but their symptoms are elaborately mentioned separately or in cluster with other diseases, such as, like malekholia, “waswasah” “mania”, “sahar”, “tawahhush”, “hizyan”, “ishque” and “khafqan”, etc. The term “izterab” is employed for anxiety in Arabic and Unani texts and therefore the word nafsani is added to izterab to specify its condition. Virtually Izterab-e-Nafsani stands for worry, worry and excessive thinking. In the standard literature of Unani system of medicine, there is no description of Izterab-e-Nafsani; however there is description of Fikr (worry) that is employed as word of hysteria. According to Unani System of medicine, Fikr may be a psychological reaction during which the Ruh-e-Haiwaniyah moves from outside to within slowly leading to coldness outside which may be felt simply [5]. Anxiety is defined as a “melancholic” disorder that is caused by excessive secretion of sauda (melancholic humour/black bile), which adversely affect the faculty that controls the nervous system, called “quwwate nafsaniya”.

~ 5 ~

Corresponding Author:
Dr. Manumula Rajaram
PG Scholar, Department of Ilmul Advia, National Institute of Unani Medicine, Bangalore, Karnataka, India

Author: Manumula Komala
PG Scholar, Department of Moalajat, National Institute of Unani Medicine, Bangalore, Karnataka, India

Author: Manchala Ashok
PG Scholar, Department of Ilaj Bit Tadbeer, National Institute of Unani Medicine, Bangalore, Karnataka, India

Author: Makula Swathi
PG Scholar, Department of Moalajat, National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, Telangana, India

Author: Nilofer Parameshwaram
PG Scholar, Department of Moalajat, National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, Telangana, India

Author: Dr. Manumula Rajaram
PG Scholar, Department of Ilmul Advia, National Institute of Unani Medicine, Bangalore, Karnataka, India
There are three main factors that adversely affect the five internal faculties of the brain, called “quwa khamsa batina”. These include, excessive secretion of black bile or melancholic humour transformation of abnormally digested safrawi (choleretic) or balgami (phlegmatic) humours in to abnormal melancholic humour and transformation in the normal quality of the melancholic humour.

Benzodiazepines, Tricyclic antidepressants (TCA’s), Monoamine oxidase inhibitors, SSRIs, azapirones are commonly used conventional medicines for treating anxiety disorders; however β –blockers, Hydroxyzone and anti-psychotic drugs are sometimes useful in treating anxiety and other medical disorders. These conventional drugs produce significant adverse effect in their long term use such as, anti-cholinergic effects, hypotension and weight gain, sleepiness and fatigue.

In Unani system of medicine, physicians have used many single and compound drugs for the management of anxiety. Hence the present is designated to evaluate the anti-anxiety effects of Kaahu and Nilofer, as the chemical constituents of these drugs have possess effects of sedation, hypnotic, headache, migraine and palpitation.

Materials and Methods
The study was conducted in NIUM, Bengaluru from June 2014 to January 2015. Before starting the experiment the research protocol was submitted for ethical clearance. The Institutional Animal Ethics Committee (IAEC) of National Institute of Unani Medicine, Bengaluru approved the protocol vide its Reg. No. IAEC 11/04/IA.

Plant materials
Kaahu – Lactuca sativa L. seeds and Nilofer (Nymphaea Alba L.) flowers were obtained by local market of Bengaluru, Karnataka. Both the drugs were authenticated by experts from Institute of Trans-Disciplinary Health Sciences and Technology, Bengaluru. The drug was dried in shade and powdered coarsely in an electric grinder. The powder was then extracted in hydroalcoholic solution (50%±50%) in the ratio of 1:5, (100 gm of powdered drug was taken into 500 ml of hydroalcoholic Hippocratic Journal of Unani Medicine 47 solution) with the help of a Soxhlet apparatus for 8 hrs. The liquid extract was then filtered and concentrated on water bath. The concentrated extract was obtained.

Experimental animal model
Twenty four Albino rats of either sex will be selected randomly. Healthy albino rats of either sex weighing 150-200gms will be included. The rats were housed in polypropylene cages, under controlled conditions of light (12/24 hour) and temperature (23±2 0C) and provided standard commercial food pellets and tap water ad libitum, under strict hygienic conditions. All the protocols and experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Dosage of the control and test drugs
The selected rats are divided into 4 groups having 6 rats in each group. The human therapeutic dose of coriander described in Unani literature, is 5-7 gm (Anonymous, 2007; Ghani, ynm). Its dose for albino rats was calculated by multiplying the higher dose of 7 gm by the conversion factor of 7 (Freirich et al., 1966) and found to be as follows; Control group will receive distilled water at a dose of 5ml/kg/b.w orally. Standard group will receive diazepam syrup at a dose of 0.11mg/kg/b.w orally. Test group 1 will receive hydroalcoholic extract of Kaahu at a dose of 51mg/kg/b.w orally. Test group 2 will receive hydroalcoholic extract of Nilofer at a dose of 266mg/kg/b.w orally.

Elevated Plus Maze (EPM)
Elevated Plus-Maze consists of two opposite open arms 50x10 cm crossed with two close arms of the same dimensions with walls 40 cm high. The arms are connected to a central square 10x10 cm2 to give the apparatus a plus sign appearance. It is elevated to a height of 40 cm from the ground. Animals were handled daily 1 week prior to the experiment and administered the drugs as per schedule. Two days before the experiment, the animals were allowed to acclimatize to the EPM apparatus. On the test day, 1 hour after oral administration of the drugs, animals were allowed to explore for elevated-plus maze. Rats were individually placed in the central square facing enclosed arm and were allowed to explore the maze for five minutes. During the next five minutes, following parameters were noted: (a) Latency of the entry of the closed arm in seconds on seventh day, (b) Number of entries in open and closed arms (entry defined as entry of 4 paws into the arm) (c) Average time spent by the animal in each arm. Animals were assessed for transfer latency, time spent in open arms and time spent in close arms and number of entries in open arm [74].

Statistical analysis of the above 4 groups were done by calculating time spent in open arms and time in close arms, number of entries in open arms and the transfer latency was recorded for each group and comparison was statistically carried out using ANOVA by standard statistical software: Vasserstat75 and Graph Pad Instat.

Results
The hydro alcoholic extracts of Kaahu (Lactuca sativa Linn) and Nilofer (Nymphaea Alba Linn) were tested in the EPM and observed a significant increase in the number of entries in open arms and time spent in open arms compared to plain control.

The following tables show the results of parameters observed in elevated plus maze paradigm for each group. The mean number of entries into open arms in distilled water treated control group was 0.5±0.223. Compared to this group, differences in the mean number of entries into the open arms in diazepam treated group (1.67±0.421), hydro alcoholic extract of kaahu treated group (2±0.365) and hydro alcoholic extract of nilofer (1.83±0.307) were statistically significant.

Table 1: Effect of Kaahu and Nilofer on number of entries in open arm by rats in Elevated Plus Maze

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Number of entries in open arm entries (Mean± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0.5±0.223</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1.67±0.421*</td>
</tr>
<tr>
<td>HAE of Kaahu</td>
<td>2±0.365**</td>
</tr>
<tr>
<td>HAE of Nilofer</td>
<td>1.83±0.307**</td>
</tr>
</tbody>
</table>

Test used: ANOVA one way with Tukey pair comparison test, ** - p < 0.01, * - p< 0.05 with respect to plain control group.
In case of percentage of total time spent in open arms, the mean percentage of total time spent in control group was 4.473±2.085. Similar to mean number of entries in open arms, the difference in percentage of total time spent in open arms in diazepam treated group (20.636±7.081), hydro alcoholic extract of kaahu treated group (22.146±2.714) and hydro alcoholic extract of nilofer (20.595±4.678) were statistically significant when compared to control group.

In the paradigm of Transfer latency (TL), there was a decrease in Transfer latency (TL) in diazepam treated group (14.181±2.082), HAE of kaahu (14.086±3.370) and HAE of nilofer (14.793±2.246) when compared to control group (28.101±3.357). It was observed statistically significant decrease in the time when compared to control group. The observations are summarized in the form of tables 1 – 3 and figures 1-3.

Table 2: Effect of Kaahu and Nilofer on time spent in open arm by rats in Elevated Plus Maze

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Time spent in Open arm entries (Mean ± SEM)</th>
<th>% time spent in open arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>4.473±2.085</td>
<td>1.491</td>
</tr>
<tr>
<td>Diazepam</td>
<td>20.636±7.081*</td>
<td>6.878</td>
</tr>
<tr>
<td>HAE of Kaahu</td>
<td>22.146±2.714**</td>
<td>7.382</td>
</tr>
<tr>
<td>HAE of Nilofer</td>
<td>20.595±4.678*</td>
<td>6.865</td>
</tr>
</tbody>
</table>

Test used: ANOVA one way with Tukey pair comparison test, ** - p < 0.01, * - p< 0.05 with respect to plain control group.

Table 3: Effect of Kaahu and Nilofer on Transfer Latency (TL) of rats in Elevated Plus Maze

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Transfer Latency (TL) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>28.101±3.357</td>
</tr>
<tr>
<td>Diazepam</td>
<td>14.181±2.082**</td>
</tr>
<tr>
<td>HAE of Kaahu</td>
<td>14.086±3.370**</td>
</tr>
<tr>
<td>HAE of Nilofer</td>
<td>14.793±2.246**</td>
</tr>
</tbody>
</table>

Test used: ANOVA one way with Tukey pair comparison test, ** - p < 0.01, * - p< 0.05 with respect to plain control group.

References
8. The ICD-10 Classification of Mental and Behavioural Disorders, Diagnostic criteria for research, World Health Organization, Geneva.
