Scientific evaluation of efficacy of waj-Acorus calamus Linn. in the management of Qillat-e-darqia (Hypothyroidism) in an animal experiment

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Abstract
AIM: The Aim of this pre-clinical study is to evaluate the efficacy of Test drug Waj (Acorus calamus) in drug induced hypothyroidism in Albino Wistar Rats.

Material & Methods: The pre-clinical study “Scientific evaluation of efficacy of Waj (A. calamus Linn.) in the management of Qillat-e-Darqia (Hypothyroidism) in Animal experiment.” 30 female albino wistar rats were used in the study. The rats were divided into 06 groups and 03 animals in each group. G1-Control group, G2-Negative group, G3-Standard group, G4-Low test dose, G5-Medium test dose group and G6-High test dose group. Except control group, all the rats were given PTU-10mg/kg/day, oral route for 30 days to induce hypothyroidism. The Hydro-alcoholic extract of Acorus calamus (HAEAC) used for the study. Toxicity study was done according to OECD 423 guidelines for Pilot study. And then the dose of test drug was fixed as Low dose (200mg/kg/bw), Medium dose (400mg/kg/bw) and High dose (600mg/kg/bw).

Results: The effect of Test drug WA (HAEAC) in PTU induced hypothyroidism, in high test dose (600mg/kg/bw) results shows the Mean ± SD of T3 is 91.667±2.517 (P < 0.01), T4 is 4.700 ± 0.200 (P < 0.01) and TSH is 26.033 ± 7.061 (P < 0.01)

Conclusion: The present pre-clinical study concluded that the test drug in high test dose (600mg/kg/bw) shows significant results in increasing the thyroid hormone levels T3 & T4 and decreasing the TSH level in PTU induced hypothyroidism in Albino wistar rats.

Keywords: Acorus calamus Linn, Qillat-e-darqia, Hydro-alcoholic extract

Introduction
- Now a day, thyroid disorders are very common throughout the world. Most commonly Hypothyroidism is an endocrine disorder (Hypothalamic-pituitary-thyroid disorder) and metabolic disorder (Iodine deficiency).
- Hypothyroidism is defined as a condition in which the thyroid gland does not produce enough thyroid hormones. Decreased secretion of thyroid hormones from the thyroid gland is called hypothyroidism [1]. This condition is known as Under-active thyroid. [6]
- Iodine deficiency also can lead to hypothyroidism because the thyroid gland needed iodine to make thyroid hormones. However, there are many other causes of hypothyroidism including thyroid gland disease (primary) and conditions of either the pituitary gland (secondary) or hypophalamus (tertiary).
- In most of the patients it starts as the glandular inflammation called thyroiditis. It eventually results in fibrosis of the gland. It occurs due to autoimmune disease (Hashimoto’s Thyroiditis) which causes destruction of the gland [7].
- Globally, about 190 million people are suffering from goiter and nearly 800 million people in developing countries are at risk. The prevalence of goiter in India is 7.3% of the total population. It is very common in females than males.
- The sub-Himalayan region is estimated that about 55 million people are suffering from endemic goiter and about 150 million are at risk. About 2.2 million are cretins and 6.6 million are having neurological deficits

Unani concept of hypothyroidism
The unani system of medicine is based on the Hippocratic theory of four humours (humoral theory) which is postulated by father of medicine Hippocrates (460 B.C), in his book “Human nature”, he mentioned that “The body contains four major kinds of humours: Blood, Phlegm, Yellow bile and black bile; A right proportion, according to quality and quantity and mixing of which (homeostasis) constitutes health.
Unright proportion and irregular distribution, according to their quantity and quality constitutes disease [30, 33, 45, 47]. According to Unani concept, any disturbance in the equilibrium of humors causes disease and therefore the treatment aims to restoring the humoral equilibrium. Unani Tibb postulates that every person from birth is endowed with a unique Mizaj (Temperament) which represents his healthy state.

The unani term for hypothyroidism is Qillat-e-darqia, it means decrease secretion of thyroid hormones from the Ghudda-e-darqia. The most common cause of Qillat-e-Darqia (Hypothyroidism) is Predominance of Phlegmatic humour due to Su-e-mizaj Barid Ratab umoomi. It leads to Excessive accumulation of phlegm in the body which results in obesity, lethargy, muscle fatigue, generalized edema and Constipation. These are the major symptoms of Hypothyroidism. Hence, it is said that there is a correlation between Su-e-mizaj Barid Ratab umoomi and Hypothyroidism.

**Test Drug:** The test drug Waj (Acorus calamus linn.) has been used from ancient times in unani system of medicine by the great unani physicians as Brain cleanser and Brain tonic, Deobstruent and resolvent. According to Hakim Najmul ghani, the test drug WAJ has been reported fot the management of “Galaganda” (Swelling of neck) which can be co-related with Hypothyroidism that is the evident from historical literature of the unani system of medicine. According to Hakim Kabeer uddin, it is used as a Desiccant of Phlegm and very useful in Brain and nerve disorders. On the basis of this I decided to work on this drug to rule out the efficacy of Waj (Acorus calamus) in the management of Qillat-e-darqia in an Animal experiment.

**Description**
Sweet Flag is a perennial herb, 30 to 100 cm tall. In habit it resembles the Iris. It consists of tufts of basal leaves that rise from a spreading rhizome. The leaves are erect yellowish-green, radical, with pink sheathing at their bases, sword-shaped, flat and narrow, tapering into a long, acute point, and have parallel veins. The leaves have smooth edges, which can be wavy or crimped. The sweet flag can easily be distinguished from Iris and other similar plants by the crimped edges of the leaves and the presence of a spadix.

**Dosage:** 1-3 grams. (Powder) [24, 25].

**Chemical constituents**
- Calamine-Active principle
- Acorn (Glucoside)-a bitter principle
- Acorin
- Starch, Tannin
- Asaryl-Aldehyde
- Heptyic acid, Palmitic acid
- Volatile Oil 1.3% (principal constituents of the Volatile oil are
- Asamyl alcohol
- Eugenol
- α Asarone & β Asarone [26, 65, 66, 68]

**Important formulations**
Majooin Nisyan, Habb-e-Bach, Mufarreh Kabir, Anqarooya Kabir, Anqarooya Sagheer

**Collection of plant material:** Dried Rhizomes of A. Calamus were collected from the local unani drugs distributer named faiz dawa saz near Charminar, Hyderabad. The plant material was identified and authenticated by botanist of CRIUM, Hyderabad.

**Macroscopic characters:** After identification and authentication of the test drug the macroscopic characters were observed and found as Rhizomes of Acorus calamus Linn.

Shape: Thumb-like branches at modes; sub-cylindrical to slightly flattened, tortuous.

Size: 1-5 cm long and 0.5-1.5 cm in width.

Colour: Light-brown with reddish-tinge to pinkish externally, buff colored internally

Fracture: Short,

Odour: Aromatic

Taste: Pungent and bitter.

**Microscopic characters**
The Rhizome of Acorus calamus Linn. was soaked in water for some time and cut section was taken and observed under microscope. TS of rhizome shows single layered epidermis; cortex composed of spherical to oblong, thin walled cells of various sizes, cells towards periphery, smaller, more or less closely arranged cells towards inner side, rounded and form a network of chains of single row of cells, enclosing large air spaces, fibro-vascular bundles and secretory cells having light yellowish-brown contents, present in this region; endodermis distinct; stele composed of round, parenchymatous cells enclosing large air space similar to those of cortex and several concentric vascular bundles arranged in a ring towards endodermis, a few vascular bundles scattered in ground tissues; starch grains simple, spherical, measuring 3-6 microns in diameter, present in cortex and ground tissue.

**Physio-chemical parameters**
- Foreign matter: Not more than 2%
- Total ash: Not more than 7%
- Acid-insoluble ash: Not more than 1%
- Alcohol-soluble extractive: Not less than 9%
- Water-soluble extractive: Not less than 16%.
- Volatile oil: Not less than 2%

**Powder study of crude drug**

**Texture:** Coarse and Heterogeneous

**Colour:** Buff coloured

**Odour:** Aromatic

**Taste:** Pungent and Bitter

The coarse powder when mixed with 10% phlorogucinol and conc. H2SO4 shows fibers, reticulate, annular vessels and simple spherical starch grains, measuring 3-6 microns in diameter.
Observation of powder and its extracts on exposure under UV light
a. Powder as such: Yellowish-cream
b. Extracts in
i. Petroleum ether: No change
ii. Chloroform: Light green
iii. Methanol: Yellowish-green
iv. Benzene: No change

Phytochemical analysis
The dried rhizome of Waj (Bach) cleaned and reduced to powder form in kharal. 20 grams of test drug was extracted with hydro-alcoholic solvents (50% Ethanol and 50% Distilled water) by Soxhlet’s Apparatus. The crude test drug material was separated and filtered. The extract was concentrated, stored and calculated. The final extract was used for preliminary phytochemical screening and other experimental purposes.

Propyl thiouracil (PTU)
Propylthiouracil (PTU) is a medication used to treat hyperthyroidism. Propylthiouracil is in the anti-thyroid family of medications. It works by decreasing the amount of thyroid hormone produced by the thyroid gland and blocking the conversion of thyroxine (T4) to triiodothyronine (T3). Propylthiouracil came into medical use in the 1940s. It is on the World Health Organization’s List of Essential Medicines, the most effective and safe medicines needed in a health system [70].

Thyroxine (Standard control drug)
The thyroid hormones are tyrosine-based hormones produced by the thyroid gland that are primarily responsible for regulation of metabolism. T3 and T4 are partially composed of iodine. A deficiency of iodine leads to decreased production of T3 and T4, enlarges the thyroid tissue and will cause the disease known as simple goitre. The major form of thyroid hormone in the blood is thyroxine (T4), which has a longer half-life than T3.

Preparation of test drug extract
The test drug used for experimental studies is in the form of hydro alcoholic extract. Extraction was prepared through the process of soxhlet apparatus. The ingredient of test drug was taken as per description of unani literature and the hydro alcoholic extract was prepared at Pharmacology laboratory, PG Dept. of Ilmul Advia, Govt. Nizamia Tibbi College (GNTC), Charminar, Hyderabad. The test drug was crushed into coarse powder and the powder was extracted in 50% distilled water and 50% alcohol by soxhlet extractor for 72 hours. The test drug extract was filtered and the solvent was evaporated on the hot plate and water bath respectively. The hydro alcoholic extract of Waj (HAEW) was dissolved in distilled water before the experiment.

Material and Methods
The present study “Scientific evaluation of efficacy of Waj in the management of Qillate-Darqia (hypothyroidism) in animal experiment.” was conducted in Animal Laboratory, Govt. Nizamia Tibbi College, Charminar, Hyderabad. This research work was approved by the IAEC (Institutional Animal Ethical Committee) Regd. No.1070/ac/07/CPCSEA dated 10.09.2016 of Govt. Nizamia Tibbi College (GNTC) Charminar, Hyderabad. The animals were purchased dated 12.10.2017 from Sainath agencies Laboratory Animals, CPCSEA No.282 dated 24-11-2000, # 1-6-197/45/D, Bapuji Nagar, Musheerabad, Hyderabad, India.

Toxic study
According to the OECD guidelines 423, I was taken 10 animals for pilot study, first I kept the animals for acclimatization for 7 days, after acclimatization as per body weight of the rat the test drug was first tested in 5 animals with dose of 1000mg/kg/bw in this dose, all animals were safe and then secondly tested in 5 animals with dose of 2000mg/kg/bw, in this dose also all animals were safe. And then the dose of test drug was fixed as Low dose (200mg/kg/bw), as Medium dose (400mg/kg/bw) and as High dose (600mg/kg/bw)

Dose: body weight of animal in grams x dose of the drug per kg ÷ 1000

Dilution: Dose of the drug as per body weight ÷ each ML of drug at the time of experiment the test drug was given by Oral route.

Experimental procedure
Animal model
“Scientific evaluation of efficacy of “WAJ” in PTU induced hypothyroidism in animal experiment”. Albino wistar female rats were given PTU-10mg/kg/day with distilled water by oral route for 30 days to induce hypothyroidism. [96] In this study, I was taken 30 female albino wistar rats weighing about 88-121 grams were used for the experiment. The rats were divided into 06 groups and 03 animals in each group as mentioned in the above table. The animals were housed under condition of controlled temperature and 12 hours day night cycle and were fed standard Rat chow and Water. The animals were randomly selected, marked to permit individual identification and was kept in their cages for a week prior to start of the experiment to allow for acclimatization to the laboratory conditions.

Group 1: The rats of Control Group received only normal saline throughout the course of the experiment.

Group 2: The rats of Negative Group received daily PTU 10mg/kg,bw for 30 days by oral route. This dose has already been shown to produce hypothyroidism.

Group 3: The rats of standard group received daily Thyroxine 5 μg/kg,bw for 30 days by oral route along with PTU 10mg/kg,bw given for 30 days.

Group 4: The rats of Low test dose group received daily HEAC 200mg/kg,bw for 30 days by oral route along with PTU 10mg/kg,bw given for 30 days.

Group 5: The rats of Medium test dose group received daily HEAC 400mg/kg,bw for 30 days by oral route along with PTU 10mg/kg,bw given for 30 days.

Group 6: The rats of High test dose group received daily HEAC 600mg/kg,bw for 30 days by oral route along with PTU 10mg/kg,bw given for 30 days. In the period of experiment, the weight of rats was checked daily and monitored their physical activity every day.
Laboratory investigations: 2ml of Blood was drawn from the Orbital sinus of rats by inserting the capillary tube into their orbital sinus for Thyroid profile (T3, T4 and TSH) and S. Cholesterol. Blood was drawn, before study i.e. 0th day, during study i.e. 15th day and the last day of study i.e. 30th day. The thyroid profile test is done by Chemi Luminescent Immuno Assay (C.L.I.A) method, and the total S. Cholesterol level test is done by CHOD-FAP method.

Observations & Results
The test drug studied for evaluation of its efficacy in the Management of hypothyroidism in Albino wistar rats. Female wistar rats weighing about 88 to 121 grams were used for the experiment.

Group I: The rats received only normal saline throughout the course of the experiment was used as Control group.

Group II: The rats of Negative Group received daily PTU 1.06 to 1.12mg (10mg/kg.bw) for 30 days by oral route. This dose has already been shown to produce hypothyroidism.

Group III: The rats of standard group received daily Thyroxine 0.53 to 0.55μg (5μg/kg.bw) for 30 days by oral route along with PTU 1.07 to 1.11mg (10mg/kg.bw) given for 30 days.

Group IV: The rats of Low test dose group received daily HEAC 17.6 to 22.4mg (200mg/kg.bw) for 30 days by oral route along with PTU 0.88 to 1.12mg (10mg/kg.bw) given for 30 days.

Group V: The rats of Medium test dose group received daily HEAC43.2 to 46.8mg (400mg/kg.bw) for 30 days by oral route along with PTU 1.08 to 1.17mg (10mg/kg.bw) given for 30 days.

Group VI: The rats of High test dose group received daily HEAC 62.4 to 72.6mg (600mg/kg.bw) for 30 days by oral route along with PTU 1.04 to 1.12 mg (10mg/kg.bw) given for 30 days.

On the 31st day all the animals were sacrificed by overdosing of anesthetic ether administered by inhalation.

Table 1: Showing detail results of Bio-chemical parameters (Thyroid profile) in Rats before statistical analysis

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Before study (0th day)</th>
<th>During study (15th day)</th>
<th>After study (30th day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rats</td>
<td>T3</td>
<td>T4</td>
<td>TSH</td>
</tr>
<tr>
<td>CG</td>
<td>R1</td>
<td>78</td>
<td>5.0</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>81</td>
<td>4.8</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>79</td>
<td>4.9</td>
<td>2.9</td>
</tr>
<tr>
<td>NG</td>
<td>R1</td>
<td>81</td>
<td>5.1</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>82</td>
<td>5.2</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>84</td>
<td>5.2</td>
<td>3.0</td>
</tr>
<tr>
<td>SG</td>
<td>R1</td>
<td>70</td>
<td>4.9</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>80</td>
<td>5.1</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>76</td>
<td>5.0</td>
<td>2.6</td>
</tr>
<tr>
<td>MG</td>
<td>R1</td>
<td>93</td>
<td>5.4</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>82</td>
<td>5.1</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>87</td>
<td>5.2</td>
<td>2.8</td>
</tr>
<tr>
<td>MG</td>
<td>R1</td>
<td>89</td>
<td>4.9</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>90</td>
<td>5.4</td>
<td>3.6</td>
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<tr>
<td></td>
<td>R3</td>
<td>92</td>
<td>5.3</td>
<td>3.1</td>
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<tr>
<td>HG</td>
<td>R1</td>
<td>78</td>
<td>4.6</td>
<td>1.9</td>
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<tr>
<td></td>
<td>R2</td>
<td>72</td>
<td>4.8</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>75</td>
<td>4.9</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Table 2: Showing values of thyroid profile in Mean ± SD after statistical analysis by Tukey Kramer multiple comparisons test-before and during study

<table>
<thead>
<tr>
<th>S. NO</th>
<th>Hormones</th>
<th>Groups</th>
<th>Before Study (Mean ± SD)</th>
<th>During Study (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T3</td>
<td>CG</td>
<td>79.333 ± 1.528</td>
<td>93.000 ± 3.606</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NG</td>
<td>82.333 ± 1.528</td>
<td>71.333 ± 3.055</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SG</td>
<td>75.333 ± 5.033</td>
<td>88.667 ± 6.110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LTDG</td>
<td>87.333 ± 5.508</td>
<td>81.667 ± 5.132</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MTDG</td>
<td>90.333 ± 1.528</td>
<td>86.667 ± 10.693</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HTDG</td>
<td>75.000 ± 3.000</td>
<td>86.667 ± 4.041</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CG</td>
<td>4.900 ± 0.1000</td>
<td>4.967 ± 0.1528</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NG</td>
<td>5.200 ± 0.1000</td>
<td>4.733 ± 0.1528</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SG</td>
<td>5.000 ± 1.000</td>
<td>4.833 ± 1.350</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LTDG</td>
<td>5.233 ± 0.1528</td>
<td>4.467 ± 0.5508</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MTDG</td>
<td>5.200 ± 0.264</td>
<td>4.333 ± 0.6110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HTDG</td>
<td>4.767 ± 0.1528</td>
<td>4.233 ± 0.5686</td>
</tr>
<tr>
<td>2</td>
<td>T4</td>
<td>CG</td>
<td>2.933 ± 0.1528</td>
<td>3.600 ± 1.480</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NG</td>
<td>3.033 ± 0.1528</td>
<td>25.167 ± 4.336</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SG</td>
<td>2.300 ± 0.3000</td>
<td>8.400 ± 3.200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LTDG</td>
<td>2.367 ± 0.4509</td>
<td>22.767 ± 3.958</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MTDG</td>
<td>3.400 ± 0.2646</td>
<td>17.300 ± 3.800</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HTDG</td>
<td>2.200 ± 0.3606</td>
<td>13.500 ± 0.7550</td>
</tr>
</tbody>
</table>
Table 3: Showing values of thyroid profile in Mena ± SD, SEM, result and P value after statistical analysis by Tukey-Kramer multiple comparisons test-after study

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean &amp; SD</th>
<th>SEM</th>
<th>Comparison</th>
<th>Mean difference &amp; result %</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>107.66 ± 2.517</td>
<td>± 1.453</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NG</td>
<td>62.667 ± 3.215</td>
<td>± 1.856</td>
<td>N vs C</td>
<td>45.00 &amp; 58.20</td>
<td>*** P &lt; 0.001</td>
</tr>
<tr>
<td>SG</td>
<td>100.67 ± 4.163</td>
<td>± 2.404</td>
<td>N vs S</td>
<td>-38.01 &amp; 60.66</td>
<td>** P &lt; 0.001</td>
</tr>
<tr>
<td>LG</td>
<td>70.333 ± 2.517</td>
<td>± 1.453</td>
<td>L vs S</td>
<td>30.33 &amp; 69.86</td>
<td>* P &lt; 0.01</td>
</tr>
<tr>
<td>MD</td>
<td>75.667 ± 4.509</td>
<td>± 2.603</td>
<td>M vs S</td>
<td>25.00 &amp; 75.15</td>
<td>** P &lt; 0.01</td>
</tr>
<tr>
<td>HD</td>
<td>91.667 ± 2.517</td>
<td>± 1.453</td>
<td>H vs S</td>
<td>9.00 &amp; 91.04</td>
<td>*** P &lt; 0.001</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>5.252 ± 0.639</td>
<td>± 0.319</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NG</td>
<td>3.267 ± 0.305</td>
<td>± 0.176</td>
<td>N vs C</td>
<td>1.952 &amp; 62.52</td>
<td>*** P &lt; 0.01</td>
</tr>
<tr>
<td>SG</td>
<td>4.867 ± 0.305</td>
<td>± 0.176</td>
<td>S vs N</td>
<td>1.60 &amp; 67.12</td>
<td>* P &lt; 0.05</td>
</tr>
<tr>
<td>LG</td>
<td>3.400 ± 0.529</td>
<td>± 0.305</td>
<td>L vs S</td>
<td>1.46 &amp; 69.85</td>
<td>* P &lt; 0.05</td>
</tr>
<tr>
<td>MD</td>
<td>3.567 ± 0.550</td>
<td>± 0.318</td>
<td>M vs S</td>
<td>1.30 &amp; 73.28</td>
<td>* P &lt; 0.05</td>
</tr>
<tr>
<td>HD</td>
<td>4.700 ± 0.200</td>
<td>± 0.115</td>
<td>H vs S</td>
<td>0.33 &amp; 96.56</td>
<td>** P &lt; 0.01</td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>2.867 ± 1.361</td>
<td>± 0.786</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>NG</td>
<td>55.700 ± 4.173</td>
<td>± 2.409</td>
<td>C vs N</td>
<td>-52.83 &amp; 94.85</td>
<td>*** P &lt; 0.001</td>
</tr>
<tr>
<td>SG</td>
<td>16.900 ± 5.549</td>
<td>± 3.204</td>
<td>S vs N</td>
<td>38.80 &amp; 69.66</td>
<td>*** P &lt; 0.001</td>
</tr>
<tr>
<td>LG</td>
<td>49.533 ± 2.686</td>
<td>± 1.551</td>
<td>L vs S</td>
<td>-32.63 &amp; 11.08</td>
<td>ns &gt; 0.05</td>
</tr>
<tr>
<td>MD</td>
<td>42.733 ± 2.902</td>
<td>± 1.676</td>
<td>M vs S</td>
<td>-25.83 &amp; 23.29</td>
<td>* P &lt; 0.05</td>
</tr>
<tr>
<td>HD</td>
<td>26.033 ± 7.061</td>
<td>± 4.077</td>
<td>H vs S</td>
<td>-9.13 &amp; 53.27</td>
<td>** P &lt; 0.01</td>
</tr>
</tbody>
</table>

T3: Hypothyroidism final result values (Table 3.A)
- In the normal control group animals table-3.A, Mean and SD was 107.66 ± 2.517.
- In the negative control group animals table-3.A, Mean and SD was 62.667 ± 3.215 and comparison of negative control group with normal control group, the mean difference was 45.00 and the P value is *** P < 0.001, resultant % is 58.20.
- In the standard control group animals table-3.A, Mean and SD was 100.67 ± 4.163 and comparison of standard control group with negative group, the mean difference was 38.01 and the P value is *** P < 0.001, resultant % is 60.66.
- In the low test dose group animals table-3.A, Mean and SD was 70.333 ± 2.517 and comparison of low test dose group with standard group, the mean difference was 7.66 and the P value is *P < 0.01, resultant % is 69.86.
- In the medium test dose group animals table-3.A, Mean and SD was 75.667 ± 4.509 and comparison of medium test dose group with standard group, the mean difference was -13.01 and the P value is **P < 0.01, resultant % is 75.15.
- In the high test dose group animals table-3.A, Mean and SD was 91.667 ± 2.517 and comparison of high test dose group with standard group, the mean difference was 29.00 and the P value is *** p < 0.001, resultant % is 91.05.

T4: Hypothyroidism final result values (Table 3.B)
- In the normal control group animals table-3.B, Mean and SD was 5.225 ± 0.639
- In the negative control group animals table-3.B, Mean and SD was 3.267 ± 0.305 and comparison of negative control group with normal control group, the mean difference was 1.95 and the P value is **P < 0.001, resultant % is 65.52.
- In the standard control group animals table-3.B, Mean and SD was 4.867 ± 0.305 and comparison of standard control group with negative group, the mean difference was -1.60 and the P value is * P < 0.05, resultant % is 67.12.
- In the low test dose group animals table-3.B, Mean and SD was 3.400 ± 0.529 and comparison of low test dose group with standard group, the mean difference was 1.46 and the P value is *P < 0.05, resultant % is 69.85.
- In the medium test dose group animals table-3.B, Mean and SD was 3.567 ± 0.550 and comparison of medium test dose group with standard group, the mean difference was 1.30 and the P value is *P < 0.05, resultant % is 73.28.
- In the high test dose group animals table-3.B, Mean and SD was 4.867 ± 0.152 and comparison of high test dose group with standard group, the mean difference was 0.166 and the P value is ** p< 0.01, resultant % is 96.56.

TSH: Hypothyroidism final result values (Table 3.C)
- In the normal control group animals table-3.C, Mean and SD was 2.867 ± 1.361
- In the negative control group animals table-3.C, Mean and SD was 55.700 ± 4.173 and comparison of negative control group with normal control group, the mean difference was -52.83 and the P value is *** P < 0.001, resultant % is 94.85.
- In the standard control group animals table-3.C, Mean and SD was 16.900 ± 5.549 and comparison of standard control group with negative group, the mean difference

Statistical analysis of data
The statistical analysis was performed using INSTAT GRAPHPAD software. Data obtained from animal experiments was expressed as arithmetic Mean ± SD and SEM. The comparison between various groups was performed by one way Analysis of Variance (ANOVA). The effect of test drug groups were compared with standard group by Tukey-Kramer Multiple Comparisons Test. P value < 0.05 was considered to be significant.
was 38.80 and the P value is *** $P < 0.001$, resultant % is 69.66.

- In the low test dose group animals table-3.C, Mean and SD was $49.533 \pm 2.686$ and comparison of low test dose group with standard control group, the mean difference was -32.63 and the P value is ns $>0.05$, resultant % is 11.08.

- In the medium test dose group animals table-3.C, Mean and SD was $42.733 \pm 2.902$ and comparison of medium test dose group with standard group, the mean difference was -25.83 and the P value is * $P < 0.05$, resultant % is 23.29.

- In the high test dose group animals table-3.C, Mean and SD was $26.033 \pm 7.061$ and comparison of high test dose group with standard control group, the mean difference was -9.13 and the P value is ** $P < 0.01$, resultant % is 53.27.

**Hypothyroidism:** Tri-iodo thyronine ($T_3$) on 15th day and 30th day CG: Control group, NG: Negative group, SG: Standard group, LG: Low dose group, MG: Medium dose group, HG: High dose group.

Fig 1: Showing results of $T_3$ before, during and after study

Fig 2: Showing results of $T_3$ in different groups after study
Hypothyroidism: Thyroxine (T₄) on 15th day and 30th day
CG: Control group, NG: Negative group, SG: Standard group, LG: Low dose group, MG: Medium dose group, HG: High dose group

Fig 3: Showing results of T₄ before, during and after study

Fig 4: Showing results of T₄ in different groups after study

Fig 5: Showing results of TSH before, during and after study
Hypothyroidism: Thyroid stimulating hormone (TSH) on 15th day and 30th day CG: Control group, NG: Negative group, SG: Standard group, LG: Low dose group, MG: Medium dose group, HG: High dose group

Discussion & Conclusion
The present Pre-clinical study was conducted to evaluate the efficacy of WAJ (BACH) in the management of Qillat-e-Darqia (Hypothyroidism) in Animal experiment. The test drug was evaluated in the present study for the effects which are reported in Unani literature and also to assess the other relative pharmacological actions. Qillat-e-Darqia (Hypothyroidism) is defined as a condition in which the thyroid gland does not produce enough thyroid hormones. This condition is known as Under-active thyroid. Iodine deficiency is the most common cause of cause of Hypothyroidism. Daily requirement of Iodine intake is 140μg/day.

Most frequently it reflects a disease of the gland itself (primary hypothyroidism) but can also be caused by pituitary disease (secondary hypothyroidism) or hypothalamic disease (tertiary hypothyroidism). It is a common disorder arising more often in women than men and increasing incidence with age, especially after the onset of middle life.

According to Unani concept, any disturbance in the equilibrium of humors causes disease and therefore the treatment aims to restoring the humoral equilibrium. The most common cause of Qillat-e-Darqia (Hypothyroidism) is Predominance of abnormal Phlegmatic humour due to Su-emizaj Barid Ratab. According to Ibn-e-Rushd, in his book Kitab-al-Kulliyat, he mentioned that all the cold and moist diseases (sard wa tar amraz) will be produce due to alteration in the quantity and quality of the Khilt-e-Balgham. Hypothyroidism is a disease of Su-e-mizaj Barid ratab and the temperament of the test drug is relatively Hot and Dry (Garm wa Khushk), so that temperament was correct the abnormal temperament of the disease, during the used of test drug in PTU induce hypothyroidism.

Waj (Bach) is commonly used in unani system of medicine as a remedy for treating various diseases from ancient times. The drug possess various medicinal properties like Munaqi-e-Dimagh (Brain Cleanser), Munzij wa Mushile-Balgham, Muhalil-e-auram (Antinflammatory), Muhafiz-e-jigar (Hepato protectve), Mudir-e-Baul (Diuretic) and Mudir-e-Haiz (Emenoggouge).

The potentially active principles could be extracted exclusively in water (50%) and alcohol (50%), therefore the hydro-alcoholic extract of the test drug was used in the present pre-clinical study. The hydro-alcoholic extract of the test drug was studied in the 3 different dosage of 200mg/kg/bw, 400mg/kg/bw and 600mg/kg/bw was administered orally in Groups 4, 5 and 6 of Albino wistar rats.

In Control Group 1st the thyroid profile (T3, T4 & STH) is normal, this group is kept as positive control group and was given only normal saline. Negative Group 2nd the thyroid hormones shows significant decrease in Thyroid hormones Resultant % (T3 – 58.20) and P Value (<0.001) and (T4 – 62.52) and P Value (<0.01) and very Significant increase in TSH, Resultant % (94.85) and P value (<0.001) as a result of PTU administration.

Standard Group 3rd shows Very significant results as Thyroid hormone levels are become very near to Normal level by increasing the Thyroid hormones Resultant% (T3 – 60.66) and P Value (<0.001) and (T4 – 67.12) and P Value (<0.05) and very Significant decrease in TSH, Resultant % (69.66) and P value (<0.001) as it is treated with Thyroxine (Standard control drug).

Group 4th, 5th and 6th treated with the test drug of Unani medicine in this study. The comparative study of different doses of test drug (HAEAC) is also desirable as the different doses may give different results and at the same time the root of drug administration is very important to get the good and effective results.

4th Group was Low test dose group (200mg/kg/bw) and the effect of test drug was mild significant in increasing thyroid hormones Resultant % (T3 – 69.86) and P Value (<0.01)
and (T4 – 69.85) and P Value (<0.05) and mild Significant in decreasing the TSH level, Resultant % (11.08) and P value (NS > 0.05).

5th Group treat as a medium test dose group (400mg/kg/bw) and the effect of test drug was moderately significant in increasing the thyroid hormones Resultant % (T3 – 75.15) and P Value (<0.01) and (T4 – 73.28) and P Value (<0.05) and mild Significant in decreasing TSH level, Resultant % (23.29) and P value (<0.05).

6th Group treated as high test dose group (600mg/kg/bw) and the effect of test drug shows very significant results in increasing the thyroid hormones Resultant % (T3 – 91.04) and P Value (<0.001) and (T4 – 96.56) and P Value (<0.01) and very Significant in decreasing TSH level, Resultant % (53.27) and P value (<0.01) to treat the PTU induce hypothyroidism in Albino wistar rats.

The present study therefore reveals that the test drug (HAEC) possesses significant effect in high test dose (600mg/kg/bw) to prevent PTU induce Hypothyroidism. The positive effect of the test drug on PTU induce hypothyroidism in experimental study is could be due to its functions of hormone releasing mechanism at the level of Hypothalamo-Pituitary-Thyroid Axis. The test drug has remarkable properties to clear depression, stress, anxiety and improves feelings of happiness, which helps to restore the balance of the thyroid hormones i.e. T3 and T4. In the present study all the animals were tolerated well, on the dose of standard and test drugs and no abnormal activities were evaluated.

Conclusion
In conclusion, the present pre-clinical study indicates significant result in drug induce hypothyroidism with hydro-alcoholic extract of Acorus calamus (HAEC) in high test dose (600mg/kg/bw) in albino wistar rats. Thus it supports traditional and ancient literature in the management of the disease. In finally the test drug was very effective in drug induced hypothyroidism and it restores the thyroid hormones in normal level, by increasing the T3 and T4 levels and decreasing the TSH level and also will be prevent from the complications of the hypothyroidism. The effect of test drug in high dose (600mg/kg/bw) of unani medicine in drug induce hypothyroidism result shows % (T3: 91.05, T4: 96.56 and TSH: 53.27).

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