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Review Article

EFFICACY OF HYDROALCOHOLIC EXTRACT OF *ACORUS CALAMUS* LINN. IN THE MANAGEMENT OF HYPOTHYROIDISM IN ALBINO WISTAR RATS – A REVIEW

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ABSTRACT

The objectives of this study were to evaluate the efficacy of hydroalcoholic extract of *Acorus calamus* Linn (HAEAC), in drug-induced hypothyroidism in albino Wistar rats, to prove the scientific integrity, credibility, and reliability of the concepts of the Unani system of medicine in the present era, to manage the disease with safe, low-cost, effective, and easily available drugs in the Unani system of medicine, and to compare the test drug with the standard control drug in the management of hypothyroidism.

Thirty female albino Wistar rats were used in the study. The rats were divided into six groups and three animals were 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 propylthiouracil (PTU)-10 mg/kg/day, oral route for 30 days to induce hypothyroidism. The HAEAC used for the study. Toxicity study was done according to Organization for Economic Co-operation and Development 423 guidelines for the pilot study. Moreover, then the dose of the test drug was fixed as low dose (200 mg/kg/bw), medium dose (400 mg/kg/bw), and high dose (600 mg/kg/bw).

The effect of test drug Waj (HAEAC) in PTU-induced hypothyroidism, in high test dose (600 mg/kg/bw) results, shows the Mean±Standard deviation of T3 is 91.667±2.517 ($p<0.001$), T4 is 4.700±0.200 ($p<0.01$), and TSH is 26.033±7.061 ($p<0.01$).

Keywords: *Acorus calamus* Linn., Unani medicine, Qillat-e-Darqia, Galaganda, Pre-clinical study.

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INTRODUCTION

Hypothyroidism is an endocrine disorder (hypothalamic-pituitary-thyroid disorder) and a metabolic disorder (iodine deficiency). It 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 [1,2].

This condition is known as under-active thyroid [5,6]. Iodine deficiency also can lead to hypothyroidism because the thyroid gland needs 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 hypothalamus (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 an 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 [11-13].

UNANI CONCEPT OF HYPOTHYROIDISM

The Unani system of medicine is based on the Hippocratic theory of four humors (humoral theory) which is postulated by the father of medicine Hippocrates (460 B.C), in his book "human nature," he mentioned that "The body contains four major kinds of humors: 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 humor 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 [37-40].

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 humor 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 [42-44,47-49].

Review literature of 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 for the management of "galaganda" (swelling of the neck) which can be correlated with hypothyroidism that is evident from historical literature of the Unani system of medicine [25-27].

According to Hakim Kabeer Uddin, it is used as a desiccant of phlegm and very useful in brain and nervine disorders. On the basis of this, the researcher decided to work on this drug to rule out the efficacy of

Waj (*A. calamus)* in the management of hypothyroidism in an animal experiment [25].

Drug description

Sweet flag *(A. calamus)* is a perennial herb, 30–100 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 [64,65].

Dosage: 1–3 g (Powder) [24,25].

Chemical constituents

Calamine and active principle, acorin (glucoside) – a bitter principle, acrotin, starch, tannin, asaryl – aldehyde, heptylic acid, palmitic acid, volatile oil 1.3%, asamyl alcohol, eugenol, α asarone, and β Asarone [26,65,66,68].

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 *A. 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.

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

Fracture: Short, Odor: Aromatic, Taste: Pungent and bitter.

Microscopic characters

The rhizome of *A. 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 toward periphery, smaller, more or less closely arranged cells toward inner side, rounded and form a network of chains of single row of cells, enclosing large air spaces, fibrovascular 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, and present in the cortex and ground tissue [64,65,68].

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 <9%
- Water-soluble extractive: Not <16%.
- Volatile oil: Not <2% [66,67].

Powder study of crude drug

- Texture: Coarse and heterogenous
- Color: Buff colored
- Odor: Aromatic
- Taste: Pungent and bitter.

The coarse powder when mixed with 10% phlorogucinol and conc. H_2SO_4 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 [64,65].

Phytochemical analysis

The dried rhizome of Waj (bach) was cleaned and reduced to powder form in kharal. 20 g of the test drug was extracted with hydroalcoholic 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.

Propylthiouracil (PTU)

PTU is a medication used to treat hyperthyroidism. PTU 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). PTU came into medical use in the 1940's. 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 the 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 goiter. The major form of thyroid hormone in the blood is thyroxine (T4), which has a longer half-life than T3 [71,72].

Preparation of test drug extract

The test drug used for experimental studies is in the form of hydroalcoholic extract. Extraction was prepared through the process of soxhlet apparatus. The ingredient of the test drug was taken as per the description of Unani literature and the hydroalcoholic extract was prepared at the pharmacology laboratory, PG Department of Ilmul Advia, Government. Nizamia Tibbi College (GNTC), Charminar, Hyderabad. The test drug was crushed into a course powder, and the powder was extracted in 50% distilled water and 50% alcohol by a soxhlet extractor for 72 h. The test drug extract was filtered and the solvent was evaporated on the hot plate and water bath, respectively. The hydroalcoholic extract of Waj was dissolved in distilled water before the experiment.

METHODS

The present study efficacy of bach – *A. calamus* Linn. In the management of hypothyroidism in albino Wistar rats – an overview, was conducted in an animal Laboratory, Government. Nizimia Tibbi College, Charminar, Hyderabad. This research work was approved by the Institutional Animal Ethical Committee Regd. No. 1070/ac/07CPCSE dated September 10, 2016, of GNCT Charminar, Hyderabad. The animals were purchased dated September 12, 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 Organization for Economic Co-operation and Development guidelines 423, I was taken 10 animals for the pilot

study, first I kept the animals for acclimatization for 7 days, after acclimatization as per the body weight of the rat the test drug was first tested in five animals with dose of 1000 mg/kg/bw in this dose, all animals were safe and then secondly tested in five animals with dose of 2000 mg/kg/bw, in this dose also all animals were safe. Moreover, then the dose of the test drug was fixed as a low dose (200 mg/kg/bw), as medium dose (400 mg/kg/bw), and as high dose (600 mg/kg/bw).

Dose
Body weight of the animal in grams×dose of the drug per kg÷1000.

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

EXPERIMENTAL PROCEDURE

Animal model
"Scientific evaluation of the efficacy of hydroalcoholic extract of sweet flag *(A. calamus* Linn.*)* in PTU-induced hypothyroidism in animal experiment." Albino Wistar female rats were given PTU – 10 mg/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 g were used for the experiment. The rats were divided into six groups and three animals in each group as mentioned in the above table. The animals were housed under condition of controlled temperature and 12-h day-night cycle and were fed standard rat chow and water. The animals were randomly selected, marked to permit individual identification, and kept in their cages for a week before the start of the experiment to allow for acclimatization to the laboratory conditions.

Group – 1: The rats of the control group received only normal saline throughout the course of the experiment.

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

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

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

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

Group – 6: The rats of the high test dose group received daily HEAC 600 mg/kg.bw for 30 days by oral route along with PTU 10 mg/kg.bw given for 30 days.

In the period of the experiment, the weight of rats was checked daily and monitored their physical activity every day.

Laboratory investigations
2 mL 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, that is, 0^{th} day, during study, that is, 15^{th} day, and the last day of study, that is, 30^{th} day. The thyroid profile test is done by the Chemi Luminescent Immuno Assay method, and the total S. cholesterol level test is done by CHOD – PAP method.

OBSERVATIONS AND RESULTS

The test drug studied for evaluation of its efficacy in the management of hypothyroidism in albino Wistar rats. Female Wistar rats weighing about 88–121 g were used for the experiment.

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

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

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

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

Group V: The rats of the medium test dose group received daily HEAC 43.2–46.8 mg (400 mg/kg.bw) for 30 days by oral route along with PTU 1.08–1.17 mg (10 mg/kg.bw) given for 30 days.

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

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

Statistical analysis of data
The statistical analysis was performed using INSTAT GRAPHPAD software. Data obtained from animal experiments was expressed as arithmetic Mean±Standard deviation (SD) and SEM. The comparison between various groups was performed by one-way analysis of variance. The effect of test drug groups was compared with standard group by Tukey-Kramer multiple comparisons test**.** $p<0.05$ was considered to be significant.

T3: Hypothyroidism final result values: (Table 3a)
In the normal control group animals Table 3a, mean and SD were 107.66±2.517

In the negative control group animals Table 3a, Mean and SD was 62.667±3.215 and comparison of the negative control group with normal control group, the mean difference was 45.00 and the p-value is ***p<0.001, and resultant % is 58.20.

In the standard control group animals Table 3a, Mean and SD was 100.67±4.163 and comparison of standard control group with the negative group, the mean difference was −38.01 the p value is ***p<0.001, and resultant % is 60.66.

In the low test dose group animals Table 3a, Mean and SD was 70.333±2.517 and comparison of low test dose group with the standard group, the mean difference was −7.66, the p value is *p<0.01, and resultant % is 69.86.

In the medium test dose group animals Table 3a, Mean and SD was 75.667±4.509, and comparison of the medium test dose group with the standard group, the mean difference was −13.01, the p value is **p<0.01, and resultant % is 75.15.

In the high test dose group animals Table 3a, Mean and SD was 91.667±2.517 and comparison of the high test dose group with the standard group, the mean difference was −29.00, the p value is ***p<0.001, and resultant % is 91.05.

T4: Hypothyroidism final result values: (Table 3b)
In the normal control group animals Table 3b, Mean and SD was 5.225±0.639

In the negative control group animals Table 3b, Mean and SD was 3.267±0.305 and comparison of the negative control group with normal

control group, the mean difference was 1.95, the p-value is **p<0.001, and resultant % is 65.52.

In the standard control group animals Table 3b, Mean and SD were 4.867±0.305 and comparison of the standard control group with the negative group, the mean difference was −1.60, the p-value is *p<0.05, and resultant % is 67.12.

In the low test dose group animals Table 3b, Mean and SD was 3.400±0.529, and comparison of the low test dose group with standard group, the mean difference was 1.46, the p-value is *p<0.05, and resultant % is 69.85.

In the medium test dose group animals Table 3b, Mean and SD was 3.567±0.550 and comparison of the medium test dose group with standard group, the mean difference was 1.30, the p-value is *p<0.05, and resultant % is 73.28.

In the high test dose group animals Table 3b, Mean and SD was 4.867±0.152 and comparison of high test dose group with the standard group, the mean difference was −0.166, the p-value is **p<0.01, and the resultant % is 96.56.

TSH: Hypothyroidism final result values: (Table 3c)

In the normal control group animals Table 3c, Mean and SD was 2.867±1.361.

In the negative control group animals Table 3c, Mean and SD was 55.700±4.173, and comparison of negative control group with normal control group, the mean difference was −52.83, the p value is ***p<0.001, and resultant % is 94.85.

In the standard control group animals Table 3c, Mean and SD were 16.900±5.549 and comparison of standard control group with the negative group, the mean difference was 38.80 and the p-value is ***p<0.001, resultant % is 69.66.

In the low test dose group animals Table 3c, Mean and SD was 49.533±2.686 and comparison of low test dose group with standard control group, the mean difference was −32.63, the p-value is ns >0.05, and resultant % is 11.08.

In the medium test dose group animals Table 3c, Mean and SD was 42.733±2.902, and comparison of the medium test dose group with standard group, the mean difference was −25.83, the p-value is *p<0.05, and resultant % is 23.29.

In the high test dose group animals Table 3c, Mean and SD was 26.033±7.061 and comparison of the high test dose group with standard control group, the mean difference was −9.13, the p-value is **p<0.01, and resultant % is 53.27.

DISCUSSION

The present experimental study was conducted to evaluate the efficacy of sweet flag *(A. calamus* Linn.*)* in the management of hypothyroidism in albino Wistar rats. 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 hypothyroidism. Daily requirement of iodine intake is 140 µg/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 humor due to Su-e-mizaj 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 produced 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 temperament was correct the abnormal temperament of the disease, during the used of test drug in PTU-induced hypothyroidism. Waj (bach) is commonly used in Unani system of medicine as a remedy for treating various diseases from ancient times. The drug possesses various medicinal properties such as Munaqi-e-Dimagh (brain cleanser), Munzij wa Mushile- Balgham, Muhalill-e-auram (antinflammatory), Muhafiz-e-jigar (hepato protective), 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 hydroalcoholic extract of the test drug was used in the present pre-clinical study. The hydroalcoholic extract of the test drug was studied in the three different dosages of 200 mg/kg/bw, 400 mg/kg/bw, and 600 mg/kg/bw was administered orally in Groups 4, 5, and 6 of albino Wistar rats.

In control group 1st, the thyroid profile (T3, T4, and STH) is normal, this group is kept as the positive control group and was given only normal saline.

Negative group 2nd, the thyroid hormones show a 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 levels 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 (hydroalcoholic extract of *A. calamus* [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.

Fourth group was low test dose group (200 mg/kg/bw), and the effect of the 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).

Fifth group treats as a medium test dose group (400 mg/kg/bw) and the effect of the 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).

Sixth group treated as a high test dose group (600 mg/kg/bw) and the effect of the 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-induced hypothyroidism in albino Wistar rats.

The present study therefore reveals that the test drug (HAEC) possesses a significant effect in high test doses (600 mg/kg/bw) to prevent PTU-induced hypothyroidism.

The positive effect of the test drug on PTU induce hypothyroidism in an 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, and anxiety and improves feelings of happiness, which helps to restore the balance of the thyroid hormones, that is, 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

The experimental study indicates significant results in drug-induced hypothyroidism with HAEAC in high test dose (600 mg/kg/bw) in albino Wistar rats. Thus, it supports traditional and ancient literature in the management of the disease. 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 (600 mg/kg/bw) of Unani medicine in drug-induced hypothyroidism result shows % {T3: 91.05, T4: 96.56, and TSH: 53.27}.

CONFLICTS OF INTREST

There is no conflicts of interest by the authors.

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