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ANTIPYRETIC ACTIVITY OF CRUDE AND AQUEOUS EXTRACT OF *GULE GHAAFIS* (*GENTIANA OLIVIERI* GRISEB) ON YEAST INDUCED PYREXIA IN ANIMAL MODEL

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ABSTRACT: *Ghaafis* (*Gentiana olivieri* Griseb) has been recommended in Classical Unani literature as an important ingredient of many antipyretic formulations. Singularly, it is advocated in both crude and extract form. The present study was conducted to investigate the antipyretic activity of crude and aqueous extract of *Gule Ghaafis* on yeast induced pyrexia in animal model. The study was carried out by the method described by Vogel. Animals were divided into six groups of six animals each. Group I received distilled water, Group II was given subcutaneous injection of 15% suspension of Brewer's yeast, Group III and IV were injected subcutaneous injection of 15% suspension of Brewer's yeast, in the dose of 10ml/kg followed by powder of *Gule Ghaafis* in the dose of 600mg/kg and 1200mg/kg, respectively. Group V and VI were given subcutaneous injection of 15% suspension of Brewer's, in the dose of 10ml/kg followed by aqueous extract of *Gule Ghaafis* in the dose of 162 mg/kg and 324mg/kg, respectively. The rectal temperature of the animals in different groups was recorded periodically for 24 hr with digital thermometer at the interval of 30min, from zero to 180 minutes. The temperature among various groups was calculated and compared by using Tukey-Kramer Multiple comparison test. The difference of mean was considered significant at $p<0.05$.The crude form of *Gule haafis* showed significant reduction in fever in higher dose and the extract of *Gule Ghaafis* in both lower and higher dose reduced fever, but the reduction in temperature was significant in higher dose. The study validated the use of *Usarae Ghaafis* (Extract of *Gule Ghaafis*) by Unani physicians in the treatment of fever. Therefore it may be concluded that extract of test drug should preferably be used particularly in the treatment of fever.

INTRODUCTION: Fever means "A body temperature above the normal range [1]. It can be caused due to abnormalities in temperature regulating centre. According to modern concept, fever is not a disease itself. It is one of the most common symptoms produced in many physiological and pathological conditions.

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But Unani system of medicine considers *Humma* (Fever) as a disease and many types of fever are described by Unani physicians such as *Hummae Youm* (Ephemeral fever), *Humma Khiltia* (Humoural fever) and *Hummae Diq* (Hectic fever). *Humma Khiltia* is based on the presence of humours and has further sub divisions.

Humma produced in absence of humours is called *Humma Yaumia*. *Hummae Diq* is a type of fever which is infiltrated in organs [2]. To control and treat fever, conventional medicine has introduced drugs like Paracetamol and Aspirin, but these drugs are reported to produce harmful side effects.

Paracetamol has been reported to cause hepatocellular damage, renal tubular necrosis in acute cases. Similarly, aspirin produces salicylism, allergy, and Rey's syndrome [3].

A number of single and compound drugs are used in the management of fever on the basis of their ascribed effects and nature of *humma* in Unani Medicine. Some plant origin Unani drugs have been reported for their Antipyretic activity, such as *Khaksi* (*Sismbrium irio)* [4], *Gilo* (*Tinospora cordifolia*), *Chiraita* (*Swertia charata*) [5] and *Ghaafis* (*Gentiana olivieri* Griseb) [6,] of them some have been scientifically proved as an effective antipyretic agent.

Ghaafis is an important drug which is used by Unani physicians as *habis* (astringent), *qatil kirme shikam* (anthelminitic), *mudir* (diuretic) and *muqawi* (tonic) [6] for long, and has also been mentioned for its antipyretic activity by various Unani scholar such as *Zakariya razi* [10], *Ibn sina* [7], *Ibn abbas majusi* [8] *Najmul gani* [9], but it has not been scientifically studied so far, for its antipyretic effect.

In Unani Medicine, most of the drugs are used in crude form, however, some drugs have also been recommended for use in extract form. Many Unani physicians have recommended the use of extract of *Ghaafis* [9]. Also from scientific view point, it may be said that drugs used in extract form will be more effective than the crude form. Therefore, present study is undertaken to evaluate the antipyretic activity of *Ghaafis* in crude and extract form in animal models.

MATERIAL AND METHODS:

The present study was carried out in the department of Ilmul Advia, National Institute of Unani Medicine (NIUM), Bangalore. Before starting the experiment the research protocol was submitted to Institutional Animal Ethics Committee (IAEC) of National Institute of Unani Medicine, Bangalore for ethical clearance Vide Reg. No. 953/C/06/CPCSEA. The experiment was stared after the approval of the protocol.

Plant Material:

Gule Ghaafis (*Gentiana olivieri* Griseb flowers) were obtained from the local crude drug market of Bangalore. The drug was identified by botanist of Raw Materials & Museum (RHMD) of Delhi Dr. H. B. Singh Vide certification reference no. NISCAIR/RHMD/Consult/-2012-13/2034/42 and a certificate for the same was issued. Voucher specimens were retained for further reference; the same were also submitted to the department of Ilmul Advia, National Institute of Unani Medicine, Bangalore.

Drugs and reagents:

Brewer's yeast (Sigma-Aldrich) and Crocin (GlaxoSmithKline) were used in the study.

Preparation and Dosage of the test Drug:

The test drug was dried in shade and powdered coarsely in an electric grinder in the pharmacy of National Institute of Unani Medicine, Bangalore. Since, both crude and whole extract of the test drug were used separately, aqueous extract was prepared by Soxhlet apparatus. Shaded dried *Gule Ghaafis* (100gm) was extracted in distilled water for six to eight hours. The filtrate was evaporated on water bath. Since the aqueous extract was used for the study, the dose of the extract was calculated with reference to the dose of air dried drug after obtaining the 27% yield percentage. The test drug was given orally in the dose of 600mg/kg and 1200mg/kg of crude form and 162mg/kg and 324mg/kg of extract dissolved in distilled water [11].

The standard drug was also given orally in the dose of 200mg/kg dissolved in distilled water [12]. A 15% suspension of Brewer's yeast which was prepared by adding 15gm of Brewer's yeast powder in 100ml of 0.9% of normal saline. The Brewer's yeast injection was given subcutaneously to the test animal in the dose of 10ml/kg [13].

Animals

The study was carried out in healthy adult albino Wistar rats of either sex; weighing 150-200 gm. Rats were procured from a registered animal breeder of Bangalore. The animals were kept in the animal house of NIUM, Bangalore and were housed in polyprophylene cages under controlled conditions of light (12/12) and temperature (23±20C) and fed with standard commercial food pellet (Hindustan Lever Ltd.) and tap water *ad libitum*, under hygienic conditions. The animals

were acclimatized to laboratory conditions for 15 days before the experiment.

Treatment groups:

Before experimentation rats were fasted overnight but water was given *ad libitum*. The experiment was conducted in six groups of rats consisting of six rats in each group. The rats were administered the crude and aqueous extracts of *Gule Ghaafis* orally in graded doses of 600, 1200mg/kg and extract 162 and 324mg/kg body weight. In pyretic rats, crude and aqueous extracts of *Gule Ghaafis* was administered orally, the model of experiment was as follows-

Group I- Distilled water 1ml/kg, orally for 24hours.

Group II- Acetaminophen orally as standard drug at the dose of 200mg/kg [12].

Group III- Powder of *Gule Ghaafis* orally at the dose of 600mg/kg.

Group IV- Powder of *Gule Ghaafis* orally at the dose of 1200mg/kg.

Group V- Aqueous extract of *Gule Ghaafis* orally at the dose of 162 mg/kg.

Group VI- Aqueous extract *Gule Ghaafis* orally at the dose of 324mg/kg.

Pyrexia induced by Brewer's yeast:

Antipyretic activity was measured by Brewer's yeast induced pyrexia in rats by the procedure described by Vogel [12] was followed for the antipyretic studies. The animals were divided into six groups having six in each. By insertion of a Digital thermometer to a depth of 2cm into the rectum the initial rectal temperatures are recorded. The fever was induced in animal by injection of 10ml/kg of Brewer's yeast suspension subcutaneously in the back below the nape of the neck. The site of injection is massaged in order to spread the suspension beneath the skin. The room temperature kept at 22-24^{0}C. Immediately after Brewer's yeast administration, food is withdrawn. 18 h post challenge, the rise in rectal temperature was recorded. The measurement is repeated after 30min.

Only those rats which showed an increase in temperature of at least 0.6^{0}C (1^{0}F) were used for further experiment. The animals receive the test or standard drug by oral administration. After the drug was administered the rectal temperature of all the rates in each group was recorded at an interval of 30, 60, 120 and 180 min post dosing [14].

Statistical analysis:

All the values are expressed as mean ±standard error of means for analyzed statistically by repeated measures ANOVA tests with Tukey-Kramer Multiple comparison test significant at $p < 0.01$ levels.

RESULTS AND DISCUSSION:

The results are presented in **Table 1**. Effect of crude form and aqueous extract of *Gule Ghaafis* and Acetaminophen (Crocin) in Brewer's yeast induced pyrexia in rats is given in **Fig. 1** and **Fig. 2** respectively. Changes in body temperature of rats treated with crude and aqueous extract of *Gule Ghaafis* and Acetaminophen (Crocin) are given in **Fig. 3** and **Fig. 4** respectively.

TABLE 1: EFFECT OF CRUDE FORM AND AQUEOUS EXTRACT OF *GULE GHAAFIS* ON BREWER'S YEAST INDUCED PYREXIA IN RATS

Name of Groups	[Mean ± SEM Rectal Temperature (in Fahrenheit)]					
		Post treated				
	Before induction	0 hr	30mnt	60mnt	120mnt	180mnt
Group I Plain control	98.96±0.27	98.96±0.27	98.73±0.15	98.11±0.15	98.13±0.22	98.63±0.20
Group II Standard Control	98.75±0.11	100.65 [a,e] ±0.18	99.40[a]±0.12	99.21[a]±0.17	99.30[a]±0.11	98.91[a]±0.18
Group III Test group (Crude form lower dose	98.95±0.20	100.48[e]±0.23	100.15[e]±0.46	100.65[b,e]±0.27	100.48[c,e]±0.42	100.23[e]±0.44
Group IV	99.40±0.20	100.61[e]±0.21	100.71[e]±0.17	100.86[b,e]±0.40	100.60[e]±0.36	100.10[e]±0.41

Test group (Crude form higher dose)						
Group V Test group (Aqueous extract lower dose)	99.51±0.60	101.08^{e}±0.41	100.96^{e}±0.38	102.03^{b,e}±0.36	101.05^{e}±0.43	102.31^{e}±0.38
Group VI Test group (Aqueous extract higher dose)	99.16±0.13	100.51^{e}±0.21	100.25^{e}±0.21	99.91^{d}±0.23	98.33^{d}±0.24	98.93^{d}±0.24

N= 6 in each group.

Test used: ANOVA repeated measure for intragroup comparison and ANOVA one way for intergroup, Comparison with Tukey-Kramer Multiple comparison test. a-p<0.01 with respect to 0hr with respecting groups, b-p<0.05 with respected standard 60mnt, c-p<0.05 with respected standard 120mnt, d-p<0.05 with respected standard 180mnt, e-p<0.05 with respect to before induction standard group.

Not significant with respect to before induction 120 and 180mnt standard. GroupVIth at 60 and 120mnt.

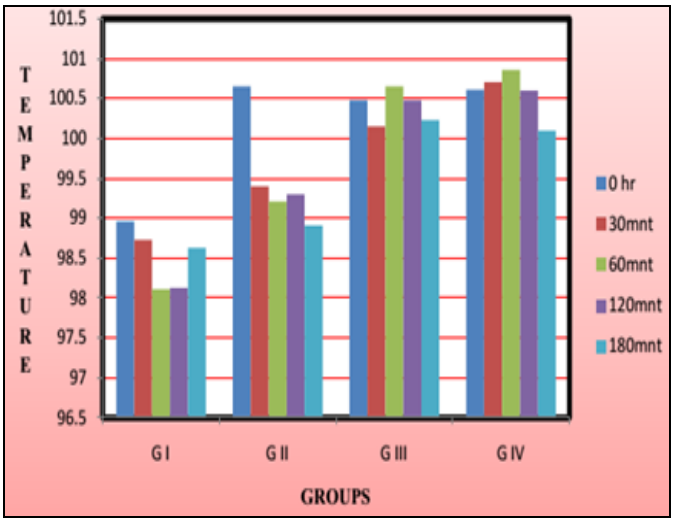

FIG. 1: EFFECT OF CRUDE FORM OF *GULE GHAAFIS* AND ACETAMINOPHEN (CROCIN) IN BREWER'S YEAST INDUCED PYREXIA IN RATS

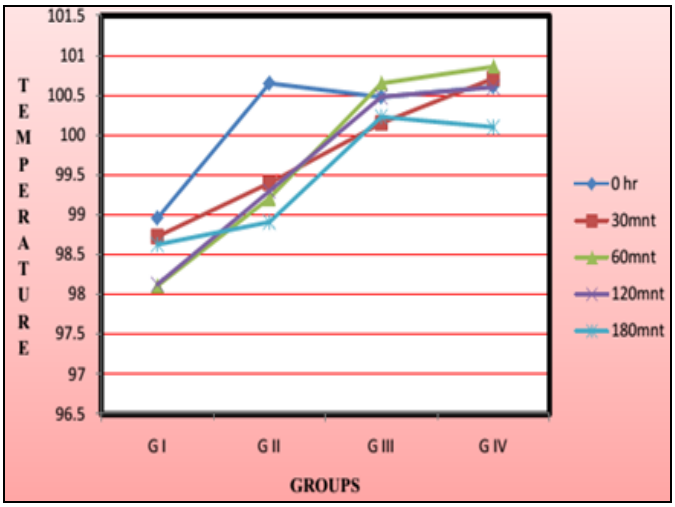

FIG. 3: CHANGES IN BODY TEMPERATURE OF RATS TREATED WITH CRUDE FORM OF *GULE GHAAFIS* AND ACETAMINOPHEN (CROCIN) IN BREWER'S YEAST INDUCED PYREXIA IN RATS

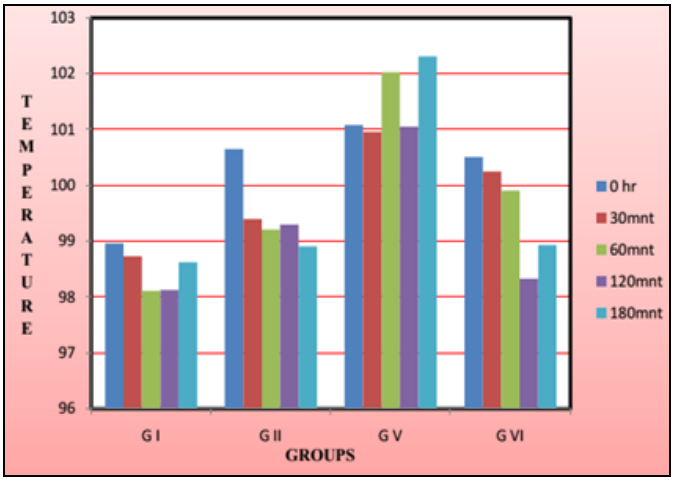

FIG.2: EFFECT OF AQUEOUS EXTRACT OF *GULE GHAAFIS* AND ACETAMINOPHEN (CROCIN) IN BREWER'S YEAST INDUCED PYREXIA IN RATS

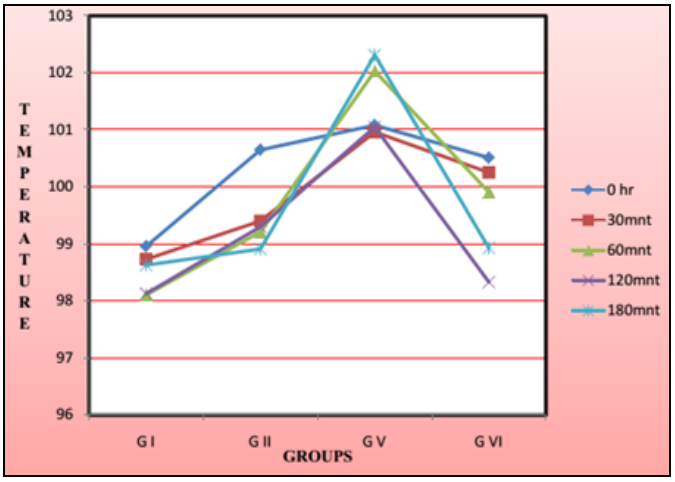

FIG. 4: CHANGES IN BODY TEMPERATURE OF RATS TREATED WITH AQUEOUS EXTRACT OF *GULE GHAAFIS* AND ACETAMINOPHEN (CROCIN) IN BREWER'S YEAST INDUCED PYREXIA IN RATS

Within group analysis of mean rectal temperature of animal groups revealed that there was no significant difference in mean rectal temperature of plain control group (Group1) at '0' hr, 30, 60, 120 and 180 minutes.

In the standard control (Group II), 18 hr after the administration of Brewer's yeast injection, the mean rectal temperature recorded at '0' hr was 100.65 ± 0.18^0F. After giving standard drug the temperature came down to 99.40 ± 0.12^0F, 99.21 ± 0.17^0F, 99.30 ± 0.11^0F and 98.91 ± 0.18^0F at 30, 60, 120 and 180 minutes, respectively. When the temperature at different intervals of time was compared to before and after induction of pyrexia, it was found that the standard drug reduced temperature significantly ($p<0.01$).

In group (Group III) After giving the test drug at the dose 600mg/kg body weight, showed the temperature came down 100.15 ± 0.46^0F to 100.23 ± 0.44^0F but when the temperature at different interval of time was compared to before and after induction of pyrexia, it was found that the test drug reduced temperature but not significant and the group IV received test drug at the dose 1200mg/kg showed the temperature came down 100.71 ± 0.17^0F to 100.10 ± 0.41^0F but when the temperature at different interval of time was compared to before and after induction of pyrexia, it was found that the test drug reduced temperature but not significant statistically ($p>0.05$).

Analysis of powder treated groups showed that there was no significant mean rectal temperature difference ($p>0.05$) found at the time interval of '0'hr, 30, 60, 120 and 180 minutes. But there was a significant difference ($p<0.05$) in mean rectal temperature observed in post-treated test group (group VI) at 120 and 180 minutes when compared with mean rectal temperature at '0'hr in the respective group. The results have shown a significant antipyretic effect ($p<0.05$) of higher dose of aqueous extract of *Gule Ghaafis* at 120 and 180 minutes in post-treated group when comparing with standard control group.

No significant antipyretic effect of *Gule Ghaafis* was observed in test group III and IV (Crude form test drug) and test group V (aqueous extract lower dose). When the groups were compared with each other it was found that the standard drug and the extract at higher dose reduced the pyrexia at 30, 60, 120 and 180 minutes. However, other groups too reduced pyrexia but the reduction was not very significant.

Antipyretic activity is commonly mentioned as a characteristic of drugs/compounds which have an inhibitory effect on prostaglandin-biosynthesis Subcutaneous injection of Brewer's yeast induces pyrexia by increasing synthesis of prostaglandin which ultimately increases the body temperature. The result seems to support the view that significant extracts has some influence on prostaglandin synthesis [15]. The result of the study indicates that the flower of *Gentiana olivier* Griseb posses antipyretic activity due to an effect on prostaglandin synthesis. The drug may act by inhibition of the production of prostaglandins. As it is well known that most of the anti-inflammatory**,** analgesic drugs possess antipyretic activity too. In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through inhibition of prostaglandin synthesis within the hypothalamus.

It is well-known that phytochemicals are responsible for many of the pharmacological activities. Flavonoids and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing the fever. Since flavonoids exhibit several biological effects such as anti-inflammatory, antimicrobial, antihepatotoxic and antiulcer activities, it is likely that the antipyretic action of *Gentiana olivier* Grseb is related to the presence of flavonoids [16]. The antipyretic activities also may be attributed to the presence of alkaloids, phenols, polyphenols, saponins, tannins, anthraquinones, steroids and especially the diterpenes [17]. The test drug contains bitter secoiridoid glycosides, olivierosides A (1), B (2) and C (3), gentiopicroside, sweroside, 6-O-β-D-glucosylgentiopicroside, swertiapunimarin, eustomoside, eustomorusside and septemfidoside [18] Furthermore, several alkaloids, fatty acids, secoiridoids, triterpenoids (oleanolic acid (OA) and ursolic acid from flowers) and bioflavonoids were isolated from the plant. Oleanolic acidic ubiquitous triterpenoid in plant kingdom, medicinal herbs, and is integral part of the human diet. OA (oleanolic

acid) is a main triterpenic acid reported in GOG flowers [19, 20]. Some of these phytochemicals may be responsible for antipyretic activity.

CONCLUSION: The present study concluded that *Gule Ghaafis* has antipyretic activity, more significant in higher dose. The study also validated the claims of Unani physicians to use *Usarae Ghaafis* (Extract of *Gentiana olivieri* Griseb) as antipyretic drug. The significant effect of extract at higher dose is also in agreement with human dose of *Usarae Ghaafis* mentioned in classical Unani literature.

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