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A comparative pharmacological evaluation of *Taila* (oil) and *Ghrita* (ghee) prepared with *Guduchi (Tinospora cordifolia)*

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Abstract

Guduchi (*Tinospora cordifolia* wild miers) is a well-known medicinal plant, which is abundantly used in different ayurvedic formulations utilizing varieties of media. The drug has properties like *Rasayana* (rejuvenating property), *Krimighna* (anthelmintics), and *Kushtghna* (used in skin disorders), as described in ayurvedic literature. *Taila* (oil) and *Ghrita* (ghee) are used as media in Ayurvedic *Sneha* (oleaginous) formulations. Both the test drugs, *Guduchi Taila* and *Ghrita*, are prescribed in *Vatrakta* (gout) and also indicated for *Kushtha* (skin disorder). With all these details, the *Guduchi Taila* and *Guduchi Ghrita* samples, prepared by using *Taila* and *Ghrita* as media, have been subjected to comparative pharmacological investigations, to assess the impact of the media on the expression of pharmacological activity. The formulations have been evaluated for immunomodulation, anti-inflammatory, and anti-stress activities. Both the formulations have been found to be active in most of the experiments, however, with the change of media, their results vary at different levels. *Taila* prepared from *Guduchi* was found to have an immunostimulating activity. The formulation prepared with *Ghrita* exhibited an anti-stress effect with an immunosuppressing activity.

Key words: *Guduchi (Tinospora cordifolia wild miers), Guduchi Taila, Guduchi Ghrita,* Immunomodulation, Anti-inflammatory, Anti-stress

Introduction

Ayurveda is the most ancient, continuously practiced healthcare discipline in the world. In ancient ayurvedic literature, a number of references are available with regard to the testing of drugs and food on animals, for evaluating their safety, before administration on human beings.[1] In the present study, the aim is to undertake a comparative pharmacological evaluation, to determine which form of the *Guduchi*[2] will be therapeutically ideal for using in the treatment of psoriasis. *Guduchi*, which belongs to the *Menispermacae* family,[3] is a well-known drug that is used in different diseases like *Jwara* (fever),[4] *Kushtha* (skin disorders),[5] *Raktapitta* (hematological disorder),[6] and so on, in different dosage forms. From them, *Ghrita*[7] and *Taila*[8] have been selected as two different media for the study. For the present study, the reference of Chakradatta (23 / 20 – 24) has been selected for the pharmaceutical and clinical trial.[8] The clinical trial has been done on the disease psoriasis.[9] Both the drugs are described in *Vatrakta adhyaya*,[7,8] but have also been indicated for the management of *Kushtha Roga*.[10] Both the drugs have been prepared with *Guduchi kwatha*,[11] *Guduchi kalka*,[12] *Ksheera*[13] and *Murcchita Taila*,[14] and *Murcchita Ghrita*.[15] The formulations have been evaluated in test models, which are supposed to have a relevance to the mechanism of action of the drugs in the treatment of psoriasis; some of them are designed on the basis of the pathophysiology of the disease.

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Materials and Methods

Go-ghrita and plain sesame oil were taken as the control, while processed *Guduchi Ghrita* and *Guduchi Taila* were taken as the test drugs for the comparative study. Group A: *Go-ghrita* as a control, Group B: *Guduchi Ghrita* as a test drug, Group C: *Tila Taila* as a control, Group D: *Guduchi Taila*. The test drugs were prepared[16] by the scholars in the Department of Rasashastra and Bhaishajya Kalpana, Institute for Post Graduate Teaching and Research in Ayurveda,Gujarat Ayurved University, Jamnagar.

The human dose for *Guduchi Ghrita* is normally given as 10 g per day. Considering the adult human dose of both *Guduchi Taila* and *Guduchi Ghrita*, the dose for the experimental study was calculated by extrapolating the human dose to animal dose based on the body surface area ratio and was 0.96 g / kg body weight for rats (Paget and Barnes).[17]

The test drugs were administered according to the body weight of the animals by the oral route, with the help of No 3 gastric catheter sleeved on over a syringe.

Animal selection

Charles Foster strain albino rats of both sexes weighing 160 – 260 g were used for the experiments. The animals were obtained from the animal house attached to the pharmacology laboratory. They were exposed to natural day and night cycles, with ideal laboratory conditions, in terms of ambient temperature and humidity. Temperature during the time of carrying out the experiment was between 20 – 30°C and the rats were fed *ad libitum* with tap water and *Amrut* brand rat pellet feed supplied by Pranav Agro Industries.

The data generated during the study was subjected to a student's *t*-test for unpaired data to assess the statistical significance.

Immunomodulation activity

Effect on humoral antibody formation

The present study was carried out to assess the comparative effect of test drugs on antibody formation against sheep red blood cells (SRBCs). The drug was administered for 10 days. On the third day of the dosing schedule, SRBC (30%) was injected intraperitoneally to the rats in a proportion of 0.5 mL / 100 g of body weight. This SRBC solution was prepared from sheep blood collected from the city slaughterhouse in a sterilized bottle containing Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% sodium chloride). The SRBCs were thoroughly washed with sterilized normal saline by centrifuging and stored in Alsever's solution in a refrigerator. The rats were sacrificed by cervical dislocation and blood was collected in separate test tubes. Blood from the same animal (sheep) was used for both sensitization and to determine the antibody titer. From the collected blood, the serum was separated and the complement in it was inactivated by incubating it in a serological water bath for 30 minutes at 55°C. Serial two-fold dilutions of the serum in sterile saline solution were made in a volume of 0.1 mL of the microtiter plate. Approximately 0.1 mL of 2% SRBC, saline-washed thrice, was added to each well of the tray. The trays were covered and placed in a refrigerator overnight. Antibody titer (hemagglutination titer) was noted the next day. The titer was converted to $\log_2$ values for easy comparison. The spleen, thymus, and lymph nodes were dissected out from the animals and their weight was also recorded. Tissues were transferred to 10% formalin solution for fixation and later on processed for histological studies.

Evaluation for the effect of test drugs on cell-mediated immunity

The rats were sensitized on the first day of drug administration by the solution of triple antigen (1 mL), normal saline (4 mL), and 10% potash alum (1 mL). The pH of the above reagent was maintained between 5.6 and 6.8 using 10% sodium carbonate. On the seventh day the initial paw volume of the right hind paw was noted and then 0.05 mL of the above solution was injected into the right hind paw. The volume of the immunological edema thus produced was measured by the volume displacement method (Bhatt *et al*. 1977). After 24 and 48 hours with plethysmography, the percentage increase over its initial value was calculated.[18] The values from the control group were compared with the test drug–administered group, to assess the cell-mediated immunity response of the drug.

Effect of the test drugs on forced swimming stress-induced hypothermia and gastric ulcers

Effect of test drugs on stress-induced, forced swimming was studied by modifying the method described by Kulkarni *et al*.[19] Twenty-four rats were selected for the study and divided into four groups of six animals each, namely, A, B, C, and D. The rats were fasted for 48 hours by keeping them in metabolic cages and on the eighth day they were forced to swim in a water (cylindrical) container having a height of 25 cm and diameter of 10 cm. The temperature of water at the time of experiment was 30°C and height of water level was 18 cm. The rectal temperature was measured before experimentation and 20 minutes after placing them in the water container, with the help of a telethermometer assembly, as an additional parameter to assess whether they possessed adaptogenic activity or not.

Observation and Results

In the present study, two sets of experimental models were employed — the first set was for evaluation of the test drugs for immunomodulation activity and the second for evaluation of the test drugs for anti-stress activity. The immunomodulatory activity was evaluated against antibody formation against SRBC and triple antigen–induced immunological edema. The anti-stress activity was evaluated against forced swimming stress–induced gastric ulceration and hypothermia. The effect of the test drugs on antibody formation against SRBC in albino rats are shown in Table 1, while the effect of the test drugs on the absolute and relative weight of the spleen of albino rats is given in Table 2. The effect of the test drugs on the absolute and relative weight of the thymus of albino rats is summarized in Table 3.

Histological study

Spleen

The organ's color is reddish-purple indicating the great content of blood. It has no ducts, unlike other abdominal organs. It is a reticuloendothelial sponge with a supporting framework of trabeculae and reticulum, and a certain amount of lymphoid tissue superadded. Larger arteries are clothed with a sheath of lymphoid tissue and malpighian bodies. Each arteriole ends abruptly in a globular mass of cells known as ellipsoids represented by an elongated collection of reticuloendothelial cells. The capillaries leaving them end in a wide marsh of splenic pulp and then gather into the sinusoids.

Scanning spleen sections from *Ghrita* control group rats under a microscope showed normal cytoarchitecture. In sections from the *Guduchi-Ghrita*–administered group, an increased proportion of white pulp was observed. In the *Tila Taila* and

Table 1: Effect of the test drug on antibody formation against SRBC in albino rats

Group	Dose g / kg	Antibody titer (Log 2 values) Mean ± SEM	% Change
Group A — *Go-ghrita*	0.96	4.15 ± 0.61	-
Group B — *Guduchi Ghrita*	0.96	4.38 ± 0.31	5.54↑
Group C — *Tila Taila*	0.96	4.84 ± 0.35	-
Group D — *Guduchi Taila*	0.96	5.77 ± 0.34*	19.21↑

*$P < 0.05$, ↑ = increase, SRBC - Sheep red blood cell

Table 2: Effect of the test drug on the absolute and relative weight of the spleen

Group	Dose g / kg	Weight of spleen			
		Absolute (mg) Mean ± SEM	% Change	Relative (mg / 100 g body weight) Mean ± SEM	% Change
Group A — *Go-ghrita*	0.96	375 ± 13		205 ± 12	
Group B — *Guduchi Ghrita*	0.96	450 ± 34*	20.32↑	225 ± 08	8.88↑
Group C — *Tila taila*	0.96	449 ± 38		222 ± 19	
Group D — *Guduchi Taila*	0.96	465 ± 36	3.44↑	232 ± 16	4.31↑

*$P < 0.05$, ↑ = Increase

Table 3: Effect of the test drug on the absolute and relative weight of the thymus

Group	Dose g / kg	Weight of thymus			
		Absolute (mg) Mean ± SEM	% Change	Relative (mg / 100 g body weight) Mean ± SEM	% Change
Group A — *Go-ghrita*	0.96	507 ± 39		254 ± 16	
Group B — *Guduchi Ghrita*	0.96	497 ± 29	4.12↓	249 ± 14	3.18↓
Group C — *Tila Taila*	0.96	522 ± 23		243 ± 14	
Group D — *Guduchi Taila*	0.96	489 ± 22	6.32↓	234 ± 07	19.03↓

↓ = decrease

Guduchi Taila–administered group, a marked increase in cellularity and white pulp proportion was observed.

Thymus

The thymus consists of epithelial and reticulum cells. In the interstices are densely packed aggregates of lymphoid cells. There are neither lymphoid follicles nor germinal centers associated with cellular pre-production. Yet the lymphoid cells of the cortex exhibit intense mitotic activity. The epithelial sheath cells constitute the barrier for antigens. The shrinking of the thymus happens at the expense of the lymphocytes.

Microscopic examination of the sections of the thymus from the control group showed normal cytoarchitecture. The thymus section from the *Guduchi Ghrita*–administered group also exhibited normal cytoarchitecture. However, sections from *Tila Taila*– and *Guduchi Taila*–administered groups exhibited a significant increase in cellularity.

Lymph nodes

A lymph node consists of a cortex containing a large number of spherical germinal centers and a medulla made up of a medullary cord separated by lymphatic sinusoids. The lymphoid tissue consists of reticulum cells, which are part of the reticuloendothelial system, and are both supporting and phagocytic. The reticulum cells are attached to a fine reticulum network and are phagocytic. The germinal centers have pale centers made up of large, more loosely packed cells, which show mitotic figures and may be regarded as lymphoblasts.

Scanning of the lymph node sections from different groups was carried out with a microscope at different magnifications. Lymph node sections from the *Ghrita* control and *Guduchi Ghrita*–administered group showed normal cytoarchitecture. In the *Tila Taila*–administered group, a slight decrease in cellularity was observed. In the *Guduchi Taila*–administered group, an increase in cellularity was observed.

Tables 4 and 5 show the effect of the test drugs on immunological edema in triple antigen–sensitized albino rats and forced swimming stress-induced hypothermia in albino rats, respectively, while the effect of the test drugs on gastric ulcer formation in the stomach of albino rats subjected to forced swimming stress is given in Table 6.

Discussion

Psoriasis is categorized under papulosquamous disorders.[20] It is a chronic, non-infectious, inflammatory skin disorder characterized by erythematous, sharply demarcated papules and rounded plaques covered by a silvery scale. Its exact etiopathogenesis is yet to be understood. Owing to this reason, its therapeutic management still remains empirical, aimed mainly at providing symptomatic relief. Owing to the paucity of information, the design of the experimental models to assess drug efficacy in a psoriatic condition also remains empirical to a large extent (Behl. 1998). At present, experimental model designing is mainly based on the probable mode of action of the drugs used in the treatment of psoriasis and the presumed etiopathogenesis.

Table 4: Effect of the test drug on the immunological edema in triple antigen–sensitized albino rats

Group	Dose g / kg	Percentage increase in paw volume			
		After 24 hours Mean ± SEM	% Change	After 48 hours Mean ± SEM	% Change
Group A — *Go-ghrita*	0.96	31 ± 12		32 ± 09	
Group B — *Guduchi Ghrita*	0.96	62 ± 07*	100↑	49 ± 07	53.1↑
Group C — *Tila Taila*	0.96	81 ± 09		72 ± 06	
Group D — *Guduchi Taila*	0.96	90 ± 13	11.11 ↑	49 ± 09	31.9↓

* $P <$ 0.05, ↑ = Increase, ↓ = Decrease

Table 5: Effect of the test drug on forced swimming stress-induced hypothermia in albino rats

Group	Dose g / kg	Stress induced hypothermia (°C) Mean ± SEM	% Change
Group A — *Go-ghrita*	0.96	10.50 ± 1.40	-
Group B — *Guduchi Ghrita*	0.96	07.90 ± 0.96	24.76↓
Group C — *Tila Taila*	0.96	07.50 ± 0.63	-
Group D — *Guduchi Taila*	0.96	05.86 ± 0.29*	21.86↓

* $P < 0.02$, ↓ = decrease

Table 6: Effect of test drugs on gastric ulcer formation in the stomach of albino rats subjected to forced swimming stress

Group	Dose g / kg	Ulcer index Mean ± SEM	% Change
Group A — *Go-ghrita*	0.96	2.3 ± 0.6	
Group B — *Guduchi Ghrita*	0.96	1.2 ± 0.3	47.82↓
Group C — *Tila Taila*	0.96	1.3 ± 0.1	
Group D — *Guduchi Taila*	0.96	1.2 ± 0.1	7.69↓

↓ = decrease

The data generated during the study showed significant potentiation of antibody formation against SRBC in the *Guduchi Taila*-administered group, while *Guduchi Ghrita* did not affect antibody formation to a significant extent. Furthermore, there was a significant increase in the cellularity of the spleen, thymus, and lymph node during the histological examination indicating the presence of a significant immunostimulation effect. In allergic skin conditions, immunosuppression is expected to provide relief, especially if the allergy is antibody-mediated. However, immunopotentiation was observed with the test sample. Hence, it is difficult to envisage the role of this activity in clinical settings. In the immunological edema, which is representative of cell-mediated immunity, a significant potentiation was observed in the *Guduchi Ghrita*-administered group, and a moderate, but statistically nonsignificant suppression was observed in the *Guduchi Taila*–administered group. Cell-Mediated Immunity (CMI) suppression could contribute to the therapeutic efficacy of the drug. It would be interesting to study the mechanism of immunomodulation observed by the test samples.

It has already been mentioned that the emotional factor plays an important role in the etiopathogenesis of several skin diseases. According to one estimate (Dutta Ray, 1998), psychological factors are responsible for skin diseases in about 10 – 15% of the cases, especially in the educationally and economically well-off sectors of the society. Anti-depressant and anti-psychotic drugs are often used for treating dermatological diseases. Stress, especially anxiety and mental trauma are considered to be important etiological factors that may cause psoriasis.[20] Owing to this reason, the test formulations were evaluated for anti-stress activity against forced swimming–induced stress; the parameters studied were stress-induced hypothermia and gastric ulceration. Furthermore, the hematological parameters were also recorded. Both the test samples, namely, *Guduchi Ghrita* and *Guduchi Taila* produced a moderate, but statistically nonsignificant decrease in hypothermia, indicating the presence of moderate anti-stress activity. Against the stress gastric ulcer, moderate attenuation was observed in the *Guduchi Ghrita*–administered group in comparison to the *Ghrita*–administered control rats. In the *Guduchi Taila*–administered group, only a marginal decrease in the severity of gastric ulceration was observed in comparison to the *Tila Taila*–administered group. It is necessary to point out here that in the *Tila Taila*-administered control group itself, the severity of ulceration was less in comparison to the *Ghrita* control group. In comparison to this control, moderate, although statistically nonsignificant decrease was observed in the *Guduchi Taila* administered group. Overall, both the *Guduchi Ghrita* and *Guduchi Taila* had a moderate and similar magnitude of activity. The activity profile indicated presence of moderate anti-stress activity at the dose level studied. The presence of this antistress activity could be one of the major contributing factors in the treatment of psoriasis.

An earlier study from this laboratory has shown that during a forced swimming stress, the following changes occur: a decrease in white blood cell (WBC) count, in the lymphocyte, granulocyte, and monocyte counts, an increase in the red blood

cell (RBC) count, increase in the level of macro RBC, increase in the mean corpuscular volume (MCV) and *hematocrit* (HCT), and a decrease in the *mean corpuscular hemoglobin concentration* (MCHC) and mercury (Hg) levels. Anti-stress drugs conceptually should reverse these changes. The data were analyzed in the above-mentioned background. In the present study, an increase in the platelet count of the *Guduchi Ghrita*-administered group and a marginal, but statistically significant decrease, in the MCV with *Guduchi Taila* are the only significant effects observed. Of them, a decrease in MCV may be indicative of the attenuation of stress. Another point that needs to be considered is that *Ghrita* (ghee) itself has a *Rasayana* (adjuvant)[2] effect, hence, it might have attenuated stress-induced changes to a considerable extent, reducing the impact of the test drugs. Whether *Tila Taila*[21] has similar properties[22] needs to be investigated. Thus, changes in the hematological parameters do not contribute in analyzing the anti-stress activity of the test formulations.

Conclusion

After thorough scanning of the results and discussion, it may be concluded that *Guduchi Taila* and *Guduchi Ghrita* produce moderate anti-stress activity, while *Guduchi Taila* is found to be immunostimulating in experimental animals.

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