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PHARMACETICO ANALYTICAL STUDYOF *'GUNJADYATAILA'* PREPARED BY USING *MURCHCHHITA* AND *AMURCHCHHITATILA TAILA* AND EVALUATION OF ITS SUB-ACUTE DERMAL TOXICITY STUDY IN ALBINO RATS- A STUDY PROTOCOL

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INTRODUCTION

Science is the intellectual process for using all of the mental and physical resources available in order to better understand, explain quantitative and predict normal as well as unusual natural phenomenon. Thus the scientific approach to understand anything involves observation, measurement of entities that can be quantities the accumulation of data, and analysis of the findings distinguished from an intuitive approach. Also science is the light thrownon silent facts which are hidden in the word-womb.

In another words, science is a gradual evolution. It is not a sudden invention Ayurveda as a science, is not an exception for it. The imperishable fundamentals of Ayurveda, which were laid down by the great sages of the ancient days are still applicable because of their scientific background. Such fundamentals must be subjected to scientific research not only to prove it certainly but also to add something new to the existing knowledge.

Ayurveda is an ancient medical science of India which helps the human body to keep fit, while providing cures from indigenous plants, animal products and minerals for various ailments.[1] Ayurveda is a complete and holistic traditional health-care system of India that contains both preventive and therapeutic aspects.[2] Ayurveda the herbal based system of medicine is now well recognized not only in India and Shrilanka but also in the Western countries.[3]

In this modern era life is full of glamour and glory hence there are many health related problem seen. In modern science there are huge numbers of drugs said for the treatment of diseases but these drugs get resistance to that particular disease. There is large number of drugs said in Ayurvedic literature.

Traditionally medicinal plant has been used for many years as topical andinternal preparation in the treatment of fungal and bacterial diseases. Medicinal plants are consider as newresources for producing agents that could act as alternative to antibiotics in the treatment of fungal and bacterial diseases.

There are various topical and systemic synthetic drugs available in the market but they have various adverse effect. In *Rastarangini Gunjadya Tail*a mention in *Kushta, Kandu.*[4] *Gunja* is herbal drug which is present in *Gunjadya Taila* belongs to sub-poisonous group. In *Gunja*abrine is present these toxic compound is harmful. Toxicity refers to chemicals within the plant being poisonous to cells. Typically exposure to toxic compounds leads to signs of subacute toxic effect. Proper evaluation of effect protects the public health. One form of toxicological assessment is animal testing of potential remedies. This study examines the use of oil in topical application. This study examine the toxic effects of *GunjadyaTaila* in albino rats at different doses for period of 28days for sub-acute toxicity study as per OECD guidelines.

Hence to provide scientific data and statistical validation the study on “Pharmacetico analytical study of *‘GunjadyaTaila’* prepared by using *Murchchhita*and *Amurchchhita TilaTaila*and evaluation of its sub-acute dermal toxicity study in albino rats” is undertaken.

1a] Need for study

1. Preparative andanalytical standards for *GunjadyaTail*are not established yet andthis study will fullfill this gap.

2. *SnehaMurchchhana*is described in *BhaishajyaRatnavali* which can improve Pharmacokinetic and Pharmacodynamics action of medicated *Taila*and *Ghee*. This study will help in understanding role of *Muchrchchhana*and its scientific bases.
3. This study is an attempt to prepare *GunjadyaTaila*with *Murchchhita* and *Amurchchhita TilaTaila*to enhance its efficacy with its pharmaceutical, analytical & sub acute dermal toxicity.
4. Skin diseases frequently occur in children and adults[5,6,7] canlead to disability, and account for the fourth leading cause ofnonfatal disease burden at the global level.[8] Various studieshave shown that skin diseases can have a major impact on the quality of life of those affected.[9] Some skin diseases, such asskin cancer and infections, are potentially life-threatening.[10] Skin diseases involve high costs for the individual and society. Many theories have been put forward with many new hypothesis describing this disorder in Ayurveda as well as in modern science; still there is enough scope to work out on its etio -pathology and management aspect of the skin diseases, because in modern medical science its management aspect remains symptomatic with troublesome side effect

2] AIM AND OBJECTIVES

2a] Aim of Study

Pharmaceutical analysis *and Sub-acute dermal toxicity Study of Gunjadya Taila* prepared by using *Murchchhita* and *Amurchchhita Tila Taila.*

2b] Objectives of study

1. To do *Murchchhana* of *TilaTaila.*
2. To do purification (*Shodhan)* of *Gunja Beeja*
3. To prepare *GunjadyaTaila*by using *Murchchhita* and *AmurchchhitaTilaTaila.*
4. To study physico-chemical parameter of *GunjadyaTaila* by traditional and modern parameter.
5. To investigate sub-acute dermal toxicity of both samples of *GunjadyaTaila* in albino rats.

3. REVIEW OF LITURATURE

a. Previous work related to the topic.

b. Review of *Gunja*(*Abrusprecatorius. Linn*).

c. Review of *Bhrungraj*(*Ecliptaalba. Hassk*)

d. Review of *TilaTaila*(seasam oil)

A. previous work related to the topic

1. Rai P. Importance of *MurchhanaSamskar*in the preparation of medicated oil – An Analytical Study. Int J AyuChem 2015 Feb 3(2): p.179-86.
2. Babu S, Kadibagil RV, Ganti N. *SnehaKalpana*: A pharmaceutical review. Journal of Biological Scientific Opion 2013; 1(3): p.271-72.
3. Veerayya R Hiremath, M.V.Subramanyam.A clinical study on *Gunjadya Tail* and *Tila Tail*Shiroabhyang in the management of darunak. Ayushdhara /july-august2016/vol-3/issue-4 ISSN:2393-9583(p)/ 2393-9591(0)
4. Banger S.K. Lahamge S.M.A comparative clinical study to evaluate role of *GunjadyaTail* And *NarikelTail*in *Darunak* w.s.rti dandruff. IntJ. ResAyu(ijrams), Sep-Oct., 2018; 1(1): 5-9.

b Review of *Gunja*(*Abrusprecatorius.Linn*)

Gunja(*Abrusprecatorius*). Awell known plant of Ayurveda under *Upvish* group is being usedextensively different formulation with great therapeutic significance and is being advocated to use in various diseases like *Indralupt* (alopecia)*Shotha* (edema)*Kushta* (skin diseases, *Kandu* (itching) *Prameh*etc after *Samskar* known as *Shodhan.* The*Gunja*seed contains number of chemical constituents like alkaloid, steroid, flavones, triterpenoids, proteins, amino acids etc). It is reported that abrusprecatorius has number of biological activity such as anti –oxidant, anticancers, anti-dibetics, nephroprotective, broncodilator, anticonvulsant, as well as anti ulcer activity.[11]

c. Review of *Bhrungraja* (*Ecliptaalba. Hassk*.)

EcliptaAlba belong family asteraceae. Ecliptaalbaadapted to a wide range of environment and found in poorly drained wet areaand canals of irrigated low land. It is reported to possess antiseptic, analgesic, antipyretic, antispasmodic and antiviral property. This plant is considered rejuvenate and good for hairand Blackening dye for hair is obtained from this plant. The leaves of EcliptaAlba are used against snake bites and scorpion stings.[12]

d. Review of *TilaTaila*(seasam oil)

Seasam is very old cultivated crop and thought to have originatedin Africa Chioroseasamone is obtained from roots of seasam haveanti-fungal activity. Seasamligane have antioxidant and health promoting activities. High amountof both sesamine and samoline were reported to increase both the hepatic mitochondrial and the perioxisomal fatty and oxidation rate.[13]

4] MATERIAL AND METHODS

4a] Source of study

1. DattatrayAyurvedRasashala, Mahatma Gandhi Ayurved College Hospital and Research centre, Salod (H) Wardha.
2. According to need study will be carried out at Certified or Standard Institute/Organization/ Lab of National Repute and as recognized or recommended by DMIMS (DU).

4b] Study Design: Pharmaceutical, Analytical and Experimental.

4c] Selection of Material

1. Drugs will be collected from the field and reliable sources.
2. Raw drugs will be verified and authenticated by Taxonomist Department of Dravyaguna M.G.A.C.H. & R.C.
3. Raw drugs will be standardized as per API.

A. The study will be carried out in the following steps

a. Process of *TailaMurchchhana*.
b. Purification of *GunjaBeeja*.
c. Preparation of *BhrungarajSwaras*.
d. Preparation of the product in 3 batches with same quantity of ingredient and same condition one with *MurchchhitTila Tail*and other with *AmurchchhitTilaTaila*
e. Sub acute dermal toxicity Study.

a. Process of *TailaMurchchhana*[14]

TailaMurchchhana will be done as per SOP

तैलंकृत्वाकटाहेदृढतरविमलेमन्दमन्दानलैस्तत् ।
तैलंनिष्क़ेनभावंगतमिहचयदाशैत्यभावंसमेत्य ॥
मंजिष्ठारात्रिलोध्रैर्जलधरनलिकै: सामलै: साक्षपथ्यै: ।
सूचीपुष्पाङ्घ्रिनीरैरुपहित्कथितैर्गन्धयोगंजहाति ॥
तैलस्येन्दुकलांशिकैकविकसाभागोडपिमूर्छाविधौ ।
येचान्ये त्रिफ़लापयोदरजनीह्रीबेरलोध्रान्विता: ॥
सूचीपुष्पवटावरोहनलिकास्तस्स्सश्चपादांशिका ।
दुर्गंधंविनिहन्तितैलमरुणंसौरभ्यमाकुर्वते ॥

भै.र. ज्वरचिकित्सा १/२६९–७०List of ingredients used for *TilaTailaMurchchhana*

Sr No	Dravya	Part to be used	Proportion
1	*Manjishtha (*Rubiacordifolia Linn*)*	Root	¼ part
2	*Haridra (Curcuma longa.Linn.)*	Rhizome	1/4 part
3	*Lodhra(* Symplocosracemosus. Roxb*)*	Stem bark	1/4 part
4	*Musta(*Cyperusrotundus.Linn)	Tuber	1/4 part
5	*Amalki*(Emblicaofficinalis.Gaertn)	Pericarp	1/4 part
6	*Bibhitaki*(Terminaliabellirica.Roxb)	Pericarp	1/4 part
7	*Haritaki*(Terminaliachebula,Retz)	Pericarp	1/4 part
8	*Vatankur(*Ficusbengalensis.Linn*)*	Leaf bud	1/4 part
9	*TilaTaila*		1 part
10	Water		4 part

a. Standard operative procedure of*Murchchhana*of *TilaTaila*

All the collected and authenticated drugs will be cleaned (to remove foreign matter)

Individually

⬇

The individual drugs will be then powdered using pulverizer.

⬇

All individual drugs will be sieved through mesh no. 80.

⬇

The required quantity of powder will be taken.

⬇

Powder of drugs will be mixed with sufficient quantity of distilled water to prepare *kalka*.

⬇

Kalka (1/4th part), *TilaTaila*(1part) and distilled water (4 parts) will be taken in clean and widemouth steel vessel.

⬇

The above mixture will be heated on *Mandagni*(low flame - gently boiling) with frequent Stirring till *SnehasiddhiLakshanas*appears.

⬇

*Taila*will be allowed to get *SwangSheeta.*

⬇

After cooling *Taila* will be filtered through doubled muslin cloth and stored in air tight glass Bottles.

⬇

MurchchhitaTila tail

b. Purification of *GunjaBeeja* [15]

GunjaBeeja will be purified as per reference with the help of *Dolayantra* in *Godugdha*

c. Preparation of *BhrungarajSwaras* [16]

BhrungrajSwaras will be prepared as per reference

d.Preparation of *GunjadyaTaila* [17]

गुंजाबीजं शिलापिष्टं कल्कीकृतमनुत्तमम् ।

तिलतैलभृंगराजपत्रनिर्यासकंतथा॥

चतुर्गुणोत्तरंचैवमानमेषांप्रकल्पयेत् ।

तैलपाकविधानेनतैलमेभिस्तुसाधितम् ॥

निहन्त्यभ्यंगयोगेन्कण्डुकुष्ठादिकामयान ।

तथादारुणकंहन्तिविविधांवातवेदनाम् ॥

र.त.त.२४/४७–४९

List of ingredients used for *GunjadyaTaila*

Sr no	dravya	praportion
1	*GunjaBeej Kalka*	1 part
2	*TilaTaila*	4part
3	*BhrungarajSwaras*	16part

Preparation of *Gunjadya Tail* by using *MurchchhitaTilaTaila*

*GunjaBeej Kalka*will be prepared

⬇

BhrungarajSwaras

⬇

Obtained*GunjaBeejkalka 1part+MurchchhitaTila Tail* (4 part) + *BhrungarajSwaras*16part

⬇

Will be taken in a wide mouth steel vessel.

⬇

Above mixture will be heated on *Mandagni*(low flame-gently boiling) with frequent stirring till the *SnehasiddhiLakshans*appears.

⬇

Taila will be allowed to get *SwangSheeta*(self-cooled)

⬇

After cooling *Tail* will be filtered through doubled muslin cloth and stored in air tight glass Bottles

⬇

Gunjadya*Taila*prepared by *MurchchhitaTilaTaila*

Preparation of *GunjadyaTaila*by using A*murchchhitaTilaTaila*

GunjaBeej Kalka will be prepared

⬇

BhrungarajSwaras

⬇

Obtained GunjaBeejKalka 1part+AmurchchhitaTilaTaila(4 part) + *BhrungarajaSwaras*16 part will be taken in a wide mouth steel vessel.

⬇

Above mixture will be heated on *Mandagni*(low flame-gently boiling) with frequent stirring tillthe *SnehasiddhiLakshans*appears.

⬇

Taila will be allowed to get *SwangSheeta* (Self cooled)

⬇

After cooling *Tail* will be filtered through doubled muslin cloth and stored in air tight glass Bottles

⬇

Gunjadya*Taila*prepared by *AmurchchhitaTilaTaila*

B Analytical study

a. Subjective Parameters of finished drugs

Description(Organoleptic characters)[18]

The prepared drug GunjadyaTaila will be evaluated by organoleptic characters.

1. *Shabda.*(Sound)
2. *Sparsha.*(Sensation)
3. *Rupa*. (Colour)
4. *Rasa.*(Taste)
5. *Gandha.*(Smell**)**

b. Objective Parameters of finished drugs

Description (Physicochemical parameters)[19]

1. Specific Gravity
2. Refractive index at 250C
3. Viscosity
4. Acid value
5. Saponification value
6. Iodine value
7. Peroxide value
8. Unsaponificable matter
9. HPTLC or GC-MS (Quantitative)

Experimental study

Animal study will be conducted according to OECD guidelines.[20]

Sub-acute dermal toxicity study

This study will be carried out according to OECD Guidelines 410 including following steps

a. Ethical clearance for study: For the conduction of dermal toxicity study on animals, prior approval will be taken from Institutional Animal Ethical Committee, DMIMSU, Sawangi Wardha.

b. Procurement and selection of animals: Required healthy young adult (at least8-10weeks old) animals male\female albino rats will be selected from animal house. Total 42Wistar strain albino rats weighing between 200-300gm, having healthy intact skin will be taken randomly for study.

a. Inclusion criteria

1. Healthy youngalbino rats of commonly used laboratory strains of either sex will be considered
2. Rats weighing about 200-300 gm will be included.
3. Rats having healthy and intact skin
4. Nuliporus.

b. Exclusion criteria

1. Pregnant and diseased rats.
2. Rats which are under trial of other experiments.

Experimental design

Scute Dermal toxicity study: [OECD GUIDLINE 410,1981]

Sub-acute dermal toxicity test will be performed according to OECD guideline 410 for testing of *Murchchhita GunjadyaTaila* (MGT) &*Amurchchhita Gunjadya Taila* (AGT).The test drug will be applied to the skin of experimental animals for period of 28 days. During the period of application of oil the animals will be observed daily to perceive signs of toxicity.

Grouping

a. Group1 – Control Group (3M,3F)

b. Group2 – Experimental group with low dose MGT(3M,3F)

c. Group 3 - Experimental group with medium dose MGT(3M,3F)

d.Group 4 - Experimental group with high dose MGT(3M,3F)

e. Group 5 - Experimental group with low dose AGT(3M,3F)

f. Group 6- Experimental group with medium dose AGT(3M,3F)

g. Group 7 - Experimental group with high dose AGT(3M,3F)

Table no. 3: Grouping of animals and dose administration in acute study.

Group	Drug	Dose	No. of Animals	Duration	Route
Group 1	Control group No Medicine	-	6(3 M +3F)	28days	-
Group 2	MGT	500 mg/kg	6(3 M +3F)	28days	Topical
Group 3	MGT	1000 mg/kg	6(3 M +3F)	28days	Topical
Group 4	MGT	2000 mg/kg	6(3 M +3F)	28days	Topical
Group 5	AGT	500 mg/kg	6(3 M +3F)	28days	Topical
Group 6	AGT	1000 mg/kg	6(3 M +3F)	28days	Topical
Group 7	AGT	2000 mg/kg	6(3 M +3F)	28days	Topical

The animals will be deprived of feed 4 hours prior of dosing and 2 hours after dosing. Drinking water will be allowed ad libitum. All the animals will be observed for 28 days after dosing. Each rat will be observed individually at least in first 30 minutes after dose administration. Then during first 24 hours with keen attentions will be given during first 4 hours.

Behaviourpattern (salivation, tremors, convulsions, diarrhoea, lethargy, sleep and coma), changes in physical appearance, and signs of illness will conduct once daily during the period, as well as any changes in fur, eyes and mucous membranes and also respiratory, circulatory, autonomicreactions to the experimental group will be observed and recorded for every single albino rat.

Histopathological study and sacrifice of animals

As per the need animals will be sacrificed after 28 days by administering mild anaesthesia. Histological examination should be performed on the preserved organs and tissues of the high dose group and the control group. The examination may be extended to animals of other dosage group. Animals in satellite group should be examined histologically with particular emphasis on those organs and tissue identified as showing effects in the other treated groupLiver, heart, kidney, intestine, stomach, brain, lungs, will be processed for histopathological studies as per the prescribed procedures.

Statistical analysis

The mean and standard deviation of the treated groups will be done by applying student t-test and ANOVA analysis.

Table no. 5: Protocol for Animal study.

Animal species	**Albino Rats**
Strain	Albino Rats
Source of Animal	Animal House, Pharmacy College, DMIMS(DU)
Average wt. of mouse	200-300 gm
No of rats	42(21 M +21F)
Age of rats	6-8 weeks
Sex of rats	50% males & 50% females
Period of Acclimatization	7 days
Temperature	22ºC (±3ºC).
relative humidity	50-60%
Route of drug administration	Local On the day before administration of the test drug, all fur will beremoved from the dorsal/flank area of the test animals (*i.e.*at least 10% of the total body surface area) by closely clipping.
Dose	500,1000,2000 mg/kg in sub acute dermal toxicity study
Observations	Animals will be observed immediately after dosing at least once during the first 30 minutes periodically during the first 24 hours, with special attention given during the first 4 hours after the beginning of the exposure period, and daily thereafter, for a total of 28 days.

METHOD OF EXPERIMENT

Procedure

After application of oil on skin, reaction will be observed. Before the test, the rats will be selected randomly and assigned to the treatment and control groups, about 10% of the body surface area will be cleared for the application of the test substance. Animals are observed for skin reactions and other signs immediately after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 2

to 6 hours after the beginning of the exposure period, and daily thereafter, for a total of 28days, Histopathological tests will bedone to compare the dermal toxicity of drugs.

Mode of administration: Topical

Duration: 28days

Comparison: All groups will be compared.

Assessment Criteria

1. General observation.

a) Changes in behaviour Pattern.

b) General appearance:

-CNS depression

-CNS stimulation

-Salivation

-Diarrhoea

-Changes in skin colour

-Hypo activity

-Passivity

-Muscle relaxation

- Muscle spasm

2. Changes in body weight (weekly)

3. Food and water consumption (weekly)

4. Faecal consistency (weekly)

5. Haematology→Hb%, Total WBC, DC.

Blood samples will be withdrawn from the posterior vena cava of each rat and will be collected in EDTA vacuumed blood collection tubes and will gently mixed immediately to mix the blood with EDTA-anticoagulant material inside the tubes for automatic and manual haematology analyses.

6. Biochemical→ SGOT, SGPT, Alkaline Phosphate, Serum creatinine, Serum urea, Serum Electrolyte → Sodium and Potassium.

Blood samples will be withdrawn from intra orbital vein from each rat and will be collected in serum vacuumed blood collection tubes.

7. Histopathology→Stomach, Jejunum, Heart, Lung, Liver, Spleen and Kidney.

Skin, liver, and kidney samples will be collected and fixed in 10% formalin for 48 hrs.

Assessment Criteria

Rating of skin Reaction for the experiment[24]

Gradation of sign or observation

Skin Reaction	Rating
A) Erythema	
○ Slight, spotty/diffuse	1
○ Moderate uniform redness	2
○ Dark red with oedema	3
○ Flery red with oedema or epidermal defect (vehicle/necrosis)	4
B) Scaling	
○ Dryness shiny	1
○ Fine scale	2
○ Moderate	3
○ Severe with large flake	4
C) Fissures	
○ No fissure	0
○ Fine cracks	1
○ Single/multiple broader fissure	2
○ Cracks with hemorrhage or exudation	3
D) Oedema	
○ No oedema	0
○ Very slight oedema	1
○ Slight oedema	2
○ (Edges of area well defined by definite raising)	
○ Moderate oedema	3
○ (Raised app. 1 mm)	
○ Severe oedema	4

(Raised more than 1 mm & extending beyond area of exposure)

RESULTS

There will be no any dermal toxicity by using *Gunjadya Taila*.

SCOPE

Main scope include quality control analytical method development and validation.

Pharma industry and its assurance completly depends on quality control and analytical study

To help overcome the problem of antimicrobial resistance. It can be clinically used on patient

CONCLUSION

Dermal toxicity will not see after its use.

Ethical Clearance: Taken from institutional ethics committee & IEAC.

Source of Funding: Self.

Conflict of Interest: Nil.

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