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Standardization of *Siddha* Formulation "*Gandhagapoora Parpam*" by Using Modern Techniques

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ABSTRACT

Introduction: Siddha system of medicine is widely being practice in Tamil Nādu and the concept pertaining to drug ingredients are from plant (Mooligai), Metals, Minerals (Thaadhu) and Animal (Jeevam) origin. Cancer is one of the prime causes of mortality increasing day by day. We are in need of therapeutic for the treatment of cancer with minimal side effects. In siddha literatures tons of various formulations given for ministration of cancer. Standardization of Siddha drug is mandatory nowadays to establish their purity and its therapeutic values.
Aim: The main aim of the present study is to systematically standardize the siddha formulation of *GANDHAGA POORA PARPAM is* taken from Siddha text. **Methods:** Physicochemical analysis such as pH, ash values, and loss on drying and etc is done and etc, Phytochemical analysis such as alkaloids, Carbohydrates and etc.
Result: According to specification given for parpam the Organoleptic character of the trial drug shows justifies the genuinity, purity and quality of the finished formulation as regards to its colour, odour, Lustreless and fine powdered texture. Physicochemical analysis reveals that pH was 8.5, Ash value was *94.43 ± 1.901%*, Acid insoluble ash was *0.4167 ± 0.07024%*, Loss on drying at 105°C was *0.3 ± 0.05568%* and etc. Phytochemical analysis gives the presence of Saponins, Betacyanin and Sugar. All the results of this study ensures that Gandhaga Poora Parpam possess therapeutic properties and for further evidence for its safety and efficacy pharmacological study has to be carried out.

Keywords: Siddha, Gandhaga Poora Parpam, Standardization, Herbo-mineral formulation, Physicochemical.

1. INTRODUCTION

In siddha system, Medicine (Marundhu) is classified into two types, Internal medicine (Ul marundhu) and External Medicine (Veli Marundhu). Among the 32 types of ul marundhu Parpam is a particular form of medicine described in siddha system of medicine.[1]Parpams are the fine particles generally prepared by calcification of purified Metals, Minerals and animal products by specific process. They are oxidized in closed crucibles in pits and with cow dung cakes for the process of pudam. In this process metals or minerals converted into sulphides or oxide by various methods.[2] Parpam is an acquired form of

nano particles and are taken besides with vehicles (Adjuvant) such as milk, ghee, honey, etc., based up on diseases. This makes easily accessible and thereby eliminates their harmful effects and enhances their biocompatibility.[3] Parpam other names are Neeru, Venneeru, Parpam. As per Siddha classical literature its Mean life is 100 years.[4] Standardization of drugs means confirmation of its identity and resoluteness of its standard and purity. Lack of quality control can affect the potency and well- being of drugs that may lead to health problems in the consumers.[5] The current study was done to analyze the physicochemical, Biochemical and phytochemical properties of Gandhaga Poora Parpam which is mentioned in *Siddha* text book *Anuboga Vaithiya Navaneedham* part-6 for the management and treatment of *Yoniputru* (Cervical Cancer), *Lingaputru, Pavuthiram*(Fistula), *Megapulligal, Megapadai, Venkuttam, Kandamalai, Meganoigal* (Venereal disease). The main ingredients of the drug are *Gandhagam* (Sulphur), *Pooram* (Hydrargyrum subchloride). *Siddhar Bhogar* classified the metals and minerals into four groups in his book *"Bhogar Karasara thurai".* They are Metals (Ulogam)-12, Toxins (Paadanam)-64, Minerals (Karasaram) -24, Hydro chemicals (Uparasam)- 120. Gandhagam or Sulphur is a Paadanam as per *Siddha* text. Sulphur is a crystalline, non-metal used in the preparation like Rasayanam, Mathirai, Mezhugu, Parpam and Chendhooram as major ingredients in Siddha Medications. RasakarPooram (Calomel) is under the classification of Panjasoodham.[6]Sulphur has anti-tumor property. Calomel also having anti-tumor property. [7]Cervical cancer is the fourth most common cancer among women globally, with an estimated 604 000 new cases and 342 000 deaths in 2020. About 90% of the new cases and deaths worldwide in 2020 occurred in low- and middle-income countries.[8] So, Peoples are searching for alternate system of medicine for the management of Cervical Cancer. *Siddhars* mentioned lots of medications for treatment of Cancer in classical siddha literatures. For the usage of people, we are in need to explore these medications with evidence based for its safety and efficacy. That's why Standardization is very much needed for further preclinical and clinical trials.

2. MATERIALS AND METHODS

Preparation of the Drug:

Selection of the Test Drug:

The test drug *"Gandhaga Poora Parpam"* is the Herbo mineral formulation which indicated in the *Siddha* literature *"Anuboga Vaithiya Navaneedham* part -6" written by Abdulla sahib, Page No -29.

Collection Of the Raw Materials:

The raw drug *Ganthagam* (Sulphur) and *Pooram* (Calomel) was procured from authenticated country shop.

The ingredients are *Vellaivengayam (*Allium cepa) procured from Koyambedu market, Chennai.

Identification And Authentication:

The raw materials were identified and authenticated by Botanist and Gunapadam experts, Government Siddha Medical college, Arumbakkam, Chennai.

Each sample has been labelled as 10035/PGG/321912108/GSMC-CH/2019-2022, 10036/PGG/321912108/GSMC-CH/2019-2022, 10037/PGG/321912108/GSMC-CH/2019-2022.

A specimen sample of each raw material has been held on to the PG Gunapadam department for future reference.

Purification of the Drugs:

1. Purification of *Pooram* (Calomel)

Purification process was done as stated by literature *"Anuboga Vaithiya Navaneedham"*part -6. RasakarPooram was soaked in breastmilk for about 3 days and then it was placed above the Mica plate. The plate was kept over the stove and the breast milk was poured slowly over the rasaka pooram for 12 hours (surukku process).

2. Purification of *Ganthagam* (sulphur):

Purification process was done as stated by literature *"Anuboga Vaithiya Navaneedham"* part -6.

The sulphur is purified by cow's milk upto 7times by the method Of Aavi pudam or Dhooma pudam. And then it was triturated with Inji charu (Ginger juice) for about 2 samam (6hrs) in a stone mortar and made it in to pellets and dried in sunlight, this procedure was repeated for three times.

Table-1 Ingredients of Gandhaga Poora parpam

Name of drugs	Quantity
Ganthagam (Sulphur)	300 grams
Pooram (Hydrargyrum subchloride)	75 grams
Vellai vengayam juice (Allium cepa)	1 liter

Standard Operative Procedure for Gandhaga Poora Parpam:

1 part of Sulphur (Ganthagam) and ¼ part of Calomel (RasakarPooram) were taken and both are Triturated in a stone mortar with White onion juice for about 3 hours (Samam) and then made into pellets, and dried in sunlight, weighed and then set aside in an airtight container. Then it was labeled as GPP.

The all studies performed at The Noble Research solution, Perambur, Chennai.

PHYSICOCHEMICAL EVALUATION

Percentage Loss on Drying

10gm of Test drug taken and it was accurately weighed in evaporating dish. The sample was dried at 105°C for 5 hours and then weighed.

Percentage loss on drying = Loss of weight of sample/Wt of the sample ×100

Determination of Total Ash

Test drug *GPP* was weighed in silica dish and incinerated at the furnace a temperature 400 ºC until it turns white in colour which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Total Ash = Weight of Ash/Wt of the crude drug taken ×100

Determination of Acid insoluble Ash

The ash obtained by total ash test of the *GPP* sample will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

Acid insoluble Ash = Weight of Ash/ Wt of the crude drug taken ×100

Determination of alcohol soluble Extractive:

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and then it was allowed to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of alcohol soluble extractive in comparison with air-dried drug.

Alcohol sol. extract= Weight of extract/Wt of the sample taken ×100

Determination of water-soluble Extractive:

Test sample was macerated with 100 ml of chloroform water in a closed flask for one day, shaking frequently for 3 to 4 times and it was allowed to cool for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of water-soluble extractive in comparison with air-dried drug.

Determination of pH:

About 5gm of test sample will be dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 mins and then subjected to pH evaluation. [9,10]

PHYTOCHEMICAL EVALUATION

This is used to test drug gives the nature of chemical constituents present in the crude drug.

Test for Alkaloids:

Mayer's Test:

To the test sample, 2ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for Coumarins:

To the test sample, 1ml of 10% sodium hydroxide was added. The presence of coumarin is indicated by the formation of yellow colour.

Test for saponins:

Foam's Test:

To the test sample, 5ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

Test for Tannins:

Fecl3 Test:

To the test sample ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of Tannins.

Test for Glycosides:

Bontrager's test:

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2ml of filtered hydrolysate, 3ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

Test for Flavonoids:

Ammonia Test:

To the test sample about 5ml of dilute ammonia solution were been added followed by addition of few drops of Conc. sulphuric acid. Appearance of yellow colour indicates the presence of flavonoids.

Test for Steroids and Triterpenoids:

Lieberman-Burchard test:

To the Chloroform solution few drop acetic anhydride was added then mixed well. 1ml of concentrated sulphuric acid was added from the sides of the test tube.

The upper layer in the test tube was turn into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of Steroids.

Appearance of red ring indicate the presence of triterpenoids.

Test for phenols:

Lead acetate Test:

To the test sample, 3ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of Phenolic compounds.

Test for Cyanins:

Test for Anthocyanin:

To the test sample, 1ml of 2N sodium hydroxide was added and heated for 5min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Betacyanin:

To the test sample, 2ml of Hcl was added and heated for 5mins at 100°C. Formation of pink colour indicates the presence of Betacyanin.

Test for carbohydrates:

Benedict's test:

To the test sample about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2minutes. A characteristic-coloured precipitate indicates the presence of sugar.

Test for Proteins:

Biuret test:

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins. [11]

BIO CHEMICAL ANALYSIS OF BASIC AND ACID RADICALS:

Analytical Investigation on Test for Acid Radicals

1. Test for Carbonates

Take 1 ml of the test solution, then add about 1 ml of concentration (conc.) HCL. Formation of brisk effervescence indicates the presence of carbonates.

2. Test for chlorides

To 2 ml of test solution, about 1 ml of silver nitrate solution was added. Appearance of White precipitate indicates the presence of chloride.

3. Test for sulphates

1 ml of the test sample was taken then add diluted H2SO4 till effervescence ceases followed by this about 1 ml of barium chloride solution was added. Appearance of white precipitate indicates the presence of sulphates.

4. Test for sulphides

To 1 ml of the test sample about 2 ml of HCL was added with slight warming the mixture.

Formation of colourless gas with the smell of rotten egg indicates the presence of sulphides.

5. Test for phosphates

Take 2 ml of test solution, it treated with 2 ml of ammonium molybdate solution followed by addition of 2ml of concentrated nitric acid. Formation of yellow precipitate Indicates the presence of phosphates.

6. Test for Fluoride and Oxalate

To 2 ml of the test solution about 2 ml of dil. acetic acid and 2ml of calcium chloride solution was added. Formation of white precipitate Indicates the presence of Fluoride/ Oxalate.

7. Test for Borates

2ml of the test solution was added with sulphuric acid and 95% alcohol followed by exposure to flame. Appearance of green flame Indicates the presence of Borates.

8. Test for Nitrates

0.5 ml of test solution heated with copper turning followed by addition of sulphuric acid.

Appearance of reddish-brown gas Indicates the presence of Nitrates

Analytical Investigation on Test for Basic Radicals

1. Test for Lead

1 ml of the test solution added with 2 ml of potassium chromate solution. Formation of yellow precipitate indicates the presence of lead.

2. Test for Arsenic

1 ml of the test solution was taken and then added with 2 ml of 10% (2N) sodium hydroxide (NaOH) solution. Formation of brownish red precipitate indicates the presence of Arsenic.

3. Test for Mercury

1 ml of the test solution added with 2 ml of 10% (2N) sodium hydroxide (NaOH) solution.

Formation of yellow precipitate indicates the presence of mercury.

4. Test for Copper

1 ml of the test solution added with 1 ml of Ammonium hydroxide (NH4OH) solution. Formation of blue precipitate indicates the presence of copper.

5. Test for Ferric

To 1 ml of test solution, about 2 ml of potassium ferrocyanide was added. Formation of blue precipitate indicates the presence of ferric.

6. Test for Ferrous

To 1 ml of test solution, about 1 ml of potassium ferric cyanide solution was added. Formation of blue precipitate indicates the presence of ferrous.

7. Test for Zinc

1 ml of the test solution added with 2 ml of sodium hydroxide (NaOH) drop wise until indication appears. Formation of white precipitate indicates the presence of Zinc.

8. Test for Silver

1 ml of the test solution was added with 1 ml of conc. HCL followed. Formation of curdy white precipitate indicates the presence of silver.

9. Test for Magnesium

1 ml of the test solution added with 2 ml of sodium hydroxide (NaOH) drop wise until indication appears. Formation of white precipitate indicates the presence of Magnesium.[12]

HEAVYMETAL ANALYSIS BY Atomic Absorption Spectrometry (AAS)

Standard: Hg, As, Pb and Cd – Sigma.

Methodology:

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption

Spectrometry (AAS) Model AA 240 series. In order to determination the heavy metals such as a mercury, arsenic, lead and calcium concentrations in the test item.

Sample Digestion:

Test sample was digested with 1mol/L HCL for determination of arsenic and mercury. Similarly for the determination of Lead and Cadmium the sample were digested with 1mol/L of HNO3.

Standard preparation:

As& Hg – 100 ppm sample in 1mol/L Hcl

Cd &Pb - 100 ppm sample in 1mol/L HNO3 [13]

MICROBIAL LOAD STERILITY TEST BY POUR PLATE METOD

Objective

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / un sterile sample (formulation) when come in contact with the nutrition rich medium it promotes the growth of the organism and after stipulated period of incubation the growth of the organism was identified by characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).

Methodology

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45ºC were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (About 10 minutes). Plates were then inverted and incubated at 37º C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU. [14]

3. RESULTS

Physicochemical Evaluation:

Fig:1. Gandhaga poora parpam

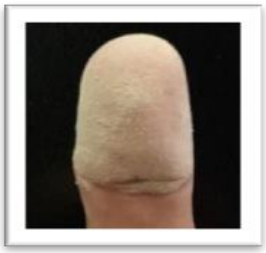

Fig: 2. Fineness -Finger ridge Analysis

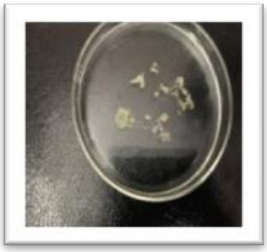

Fig: 3 Float on Water Test deposit

Table – 2 Confirmatory Specification for Parpam

Parameter	Observation for GPP
Fineness	Confirms the standard for fineness as per the particle size analysis and flow property of the sample
Float on Water	Confirms the test
Smokeless	Confirms the test
Taste less	Confirms the property
Lusterless	Confirms the property

Table -3 Results of physicochemical evaluation of Gandhaga Poora Parpam

S.no	Parameter	Result
1.	Organoleptic Characters Color Odor Touch Taste Appearance Flow property	 Whitish grey None Soft Bitter Powder Free flowing
2.	pH	8.5
3.	Loss on Drying at 105 °C (%)	0.3 ± 0.05568
4.	Total ash (%)	94.43 ± 1.901
5.	Acid insoluble ash (%)	0.4167 ± 0.07024
6.	Water Soluble Extractive (%)	9.167 ± 0.5132
7.	Alcohol Soluble Extractive (%)	0.18 ± 0.03
8.	Solubility	+ve in Ethanol, Water, DMSO.

QUALITATIVE PHYTOCHEMICAL EVALUATION OF GPP:

The result of phytochemical evaluation reveals the presence of biologically significant phytochemical such as Saponins, Sugar, Betacyanin.
The results were tabulated in table 4.

Table-4 Qualitative phytochemical Analysis of GPP:

Test	Observation
Saponins	+
Sugar	+
Betacyanin	+

+ indicates positive.

BIOCHEMICAL ANALYSIS OF GPP:

Bio chemical analysis is tabulated in table 5.

Table -5 Results of Biochemical Analysis of GPP

S. No	Parameters	observation	Result
1.	Test for Sulphates	Presence of white precipitate	+
2	Test for Nitrates	Presence of reddish-brown color	+
3	Test for Carbonates	Presence of brisk effervescence	+
4.	Test for Mercury	Presence of Yellow precipitate	+

HEAVY METAL ANALYSIS by Atomic Absorption Spectrometry (AAS):

The present study shows that the trial drug has a significant level of metals such as Lead, Arsenic, Mercury and Cadmium and the result was tabulated in table 6

Table 6- Heavy Metal Analysis

Name of the Heavy Metal	Absorption Max A Max	Result Analysis	Maximum Limit
Lead	217.0nm	BDL	10ppm
Arsenic	193.7nm	BDL	3ppm
Cadmium	228.8nm	BDL	0.3ppm
Mercury	253.7nm	0.52ppm	1ppm

BDL – Below Detection Limit

RESULT FOR MICROBIAL CONTAMINATION:

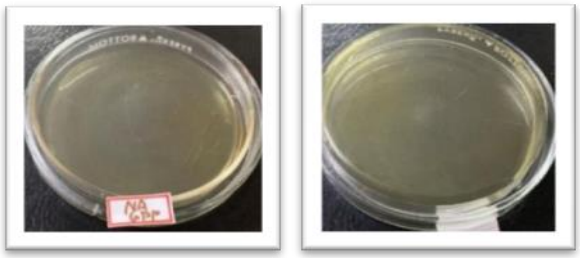

Fig: 4 Sterility test by pour plate method

The result of microbial contamination was tabulated in table 7. The table given below.

Table 7- Results for microbial contamination

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

The above table evidently prove that the test drug Gandhaga Poora parpam was free from viable microorganism. Hence the sterile drug maintains the efficacy and potency of the test drug.

4. DISCUSSION

Organoleptic property of the GPP as regards to its Whitish grey colour, none odour, very fine in nature, soft in touch, justifies the genuinity, purity and quality of the finished formulation regard to its colour, odour, fine powdered texture. Due to its small size, a drug shows increase solubility. Dissolution of solute in solvent to give a homogenous system, is one of the essential parameters to achieved concentration of drug in circulation for proper pharmacological

response. Its fineness will help to enhance the bioavailability. Lustreless will shows the proper calcination process. So that it does not cause any toxic effects.[15] Having the pH 8.5 slightly Alkaline, the pH of the test drug denotes that it is alkaline in nature. So that, in the oral administration, the drug will get ionized in stomach and absorbed in intestine and sent directly to the portal system.[16] GPP showed that Loss on drying was *0.3±0.05568*, low moisture content present in the prepared medicine. According to Ayurveda pharmacopoeia of India the LOD value is preferred to be 1 or less than 1. Thus, low moisture content could get maximum stability and better shelf life. The percentage of loss on drying was within acceptable range to thus implying that the formulation can be hoarded for a long period and would not easily be attacked by microbes. Total Ash was 94.43 ± *1.901*, Ash values are helpful in determining the quality and purity of crude drugs. Acid insoluble ash was *0.4167 ± 0.07024*, which shows the purity of the test drug. Water soluble Extractive was *9.167 ± 0.513*. It indicates the existence of sugar, acids. Alcohol soluble Extractive was *0.18 ± 0.03*. This drug GPP soluble in ethanol, water and *DMSO*.[17] There are 150 types of natural saponins coming under 100 families have been found to possess considerable anti-cancer properties.[18] Saponins display various organic activities as well as anti-tumour activity. Recently in-depth research has been focused on developing saponins for tumour therapies.[19] The cytostatic activity of diosgenyl saponins was evaluated in MCF-7 breast cancers and HeLa cervical cancer cells.[20] Structural moderation in the sugar moiety, involving oxazoline ring formation, increase the antineoplastic activity in terms of apoptosis induction and genotoxicity. It can be concluded that chemical moderation at the c3 position is a good method to increase the activity against cancer cells in vitro.[21] Betacyanin pigment described to have a significant free-radical scavenging activity, they also exhibited anticancer, anti-inflammatory and chemo preventive effects.[22] In this drug GPP the minerals Carbonates, Nitrates and Mercury are present. Carbonates is a salt of carbonic Acid. Carbonates and carbamate derivatives of 4-demethylpencolamedine has been perceived to be an active anti-tumour agent in mouse human xenograft tumour models.[23] $CacO_3$ nanoparticles have exhibited promising potential as drug carriers targeting cancer tissues and cells.[24] Increased plasma nitrate levels were also observed in response to endotoxin and to recombinant human tumour necrosis factor. Plasma nitrate concentrations may be a sensitive indication of the antitumour response.[25] Sulphated polysaccharides was derived using the chlorosulfonic acid-pyridine method. It exerts its antitumour activity by inducing apoptosis in tumour cells and the secretion of NO and TNF-α.[26] Mercury in its sulphide form have not toxic effects in body and shows anti-cancer activity.[27] The PattuKaruppu formulation contains mercurial it was very effective only to cancerous cells (MCF-7) and it is less toxic to normal cells.[28] The contamination of herbal drugs by microorganism not only cause bio disintegration but also bringdown the potency of drugs. This drug GPP reveals the absence of specific pathogen. Hence it ensures the good quality and manufacturing of GPP. The sterility of drug maintains the efficacy and potency of the test drug.[29] The extract of Allium cepa L. is often used as supportive therapy for cancer that includes a potential source of antitumour properties. Cytostatic from a crude extract of Allium cepa L. against human colon cancer (WiDr) cells.[30]

5. CONCLUSION

This study ensures that GPP has all properties of a parpam, specified in the plim guidelines. As particle size of GPP is nano in size, it will be quickly absorbed. Inorganic nanoparticles have received increased attention in the recent past as potential diagnostic and therapeutic systems in the field of oncology. Inorganic ultra-small particles have exhibit success in

imaging and treatment of tumours both test tube and living cells. So, it may helpful in the treatment of cancer. The present pharmaceutical studies reveal that GPP possess therapeutics substances. Clinical trial has to be done to validate its safety and efficacy in human after the toxicology and pharmacology studies.

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Ethical Approval: Approved

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