MATERIALS AND METHODS
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A) The test material, Suvarnamakshikadiwati in the form of powder was obtained from an Ayurvedic pharmaceutical firm. This firm is named by Hariparashuram Aushadhalaya. Vaidya Khadiwale is incharge of it and under his supervision and guidance compounds are prepared. This compound preparation contains following ingredients and each ingredient is processed separately as per Ayurvedic methodology. (Details are covered in review).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUVARNAMAKSHIK BHASMA</td>
<td>100 mg</td>
</tr>
<tr>
<td>ABHRAK BHASMA</td>
<td>20 mg</td>
</tr>
<tr>
<td>SHILAJIT</td>
<td>20 mg</td>
</tr>
<tr>
<td>VANSHLOCHAN</td>
<td>20 mg</td>
</tr>
<tr>
<td>SHRING BHASMA</td>
<td>20 mg</td>
</tr>
<tr>
<td>LOHA BHASMA</td>
<td>20 mg</td>
</tr>
<tr>
<td>ARJUNA SAL</td>
<td></td>
</tr>
</tbody>
</table>

For this work material (test compound) was provided in one lot of about 3 kg in powder form. For all experimental work the same test compound was used.

B) All the chemicals used for different studies were of the G.R. grade or pure standard drugs and powders were used for comparison or for control animals.

C) Test animals used for experimental work were rats and dogs.
Rats: Healthy adult male albino Rats of the Wister strain, bread in animal house of B.J. Medical college, Pune were used. Age and weight of the animals used for different studies were taken into consideration. The number of animals used in each group were at least six in each group.

The animals were housed in clean metal cages having wire mesh at the top. Rice husk (Paddy) was used as their bedding. Rats were provided routine normal diet containing wheat flour, milk in fixed proportion with addition of vit E.

The environmental temperature, where the animals were housed, was maintained between 19° to 25° degree centigrade. The humidity was between 30% to 70%. As the lighting was artificial, the sequence of 12 hours light, followed by 12 hours darkness was maintained.

A minimum seven days before starting the experiment was considered as the period of equilibration.

All the animals were identified uniquely so that no two animals had the same marking by picric acid.

Dogs

Adult mongrel dogs, of either sex were procured from the Pune Municipal Corporation. They were housed singly in animal house rooms of B.J. medical college Pune. They were observed over a period of 10 days to confirm that they are Rabies free and safe to use for study, also animals were aclimatized for at least one week.
D) Methods used for oral administration

For Rats, oral feeding was done by using catheter of 10 inch length, catheter was inserted gently into stomach by lubricating it with liquid paraffin. To the catheter syringe was fitted for administration of test compound.

Intravenous drug administration in Dogs was done through femoral vein. For oral feeding in dogs, rubber catheter was inserted directly into stomach in anesthetised animals by using laryngoscope.

E) Instruments and apparatus

All the glassware used was of Borosil quality. Glassware and small surgical instruments were thoroughly washed with water and soap and dried in oven. The instruments were dipped into savolone solution for aseptic purpose.

For our experimentation we used following instruments

a) Convulsiometer
b) Analgesiometer
c) Swimming apparatus
d) Blood Pressure recorder
e) Atomic absorption spectrophotometer
f) Haemoglobinometer

ANALGESIOMETER

Analgesiometer of TECHNO make was used to evaluate analgesic activity by radiant heat method. This instrument consists of a nichrome wire, which can be heated by passing varying strengths of electric current through it. An ammeter, showing 0 to 0.5
amperes indicates the strength of current which can be adjusted with the knob. The base of the tail of animal is placed on a small platform just above the nichrome wire. The platform is cooled by a water jacket, so as to prevent any transfer of heat by conduction. The time taken for the animal to produce a tail flick in response to the radiant heat coming out when the wire starts heating up, is measured in seconds.

ELECTRO CONVULSIOMETER

Electro convulsiometer (TECHNO) was used to induce convulsions by electrical shock. This instrument offers multiple choice for different strengths and duration of stimulus. Stimulus strength can be adjusted by positioning three different knobs. The range offered is from 0.25 to 360 milliamperes. The timer switch can provide a duration of the stimulus from 1 to 10 milliseconds. The shock is delivered by pressing down a switch, which is brought back to the reset position before every shock so as to avoid accidental premature shocks or multiple shocks in succession.

HAEMOGLOBINOMETER

Principle close comparison of a known pigment (haematin) which is formed an addition of acid to (Hb) haemoglobin. This is very small and convenient instrument, which is routinely used to estimate blood haemoglobin. This contains small 0.5 mm diameter tube which is marked on one side with 100-140 divisions.

This tube is placed on such a platform, having two sides, on one side a standardised yellowish brown glass plates are
attached. After taking blood, this is diluted with hydrochloric acid (0.1 N) till it matches with the yellow brown colour of the standard plate. Micro pipettes are supplied of small size, with which exactly 0.02 ml of blood can be collected from finger prick for estimation of Hb content. 100% = 14 gms Hb per 100 cc PATGMT- 549919.
METHODS IN DETAIL

1) TOXICOLOGICAL STUDIES

1.1 Acute Toxicity study in Rats

The purpose of this study was to determine the oral LD50 of the test compound that is SUVARNAMAKSHIKADIWATI.

For this study, male albino rats weighing between 200-250gms were selected. Rats were allowed to access the food and water ad libidum (as given routinely to rats in the animal house of B.J. Medical College Pune). This study was carried out in 2 parts.

Part I - After weighing and proper marking with picric acid, animals were divided into 5 groups as follows and were administered the test compound.

No of animals in each gr = 6

Control I - 2ml Gum tragacanth
Group II - Test compound 10 mg/100gm
Group III - Test compound 40 mg/100 gms
Group IV - Test compound 160 mg/100 gms
Group V - Test compound 640 mg/100 gms

In part II study following were the groups.

Control I 2 ml Gum tragacanth
Group II Test compound 640 mg/100 gms
Group III Test compound 1280 mg/100 gms
Group IV Test compound 2560 mg/100 gms

In part I and II Test compound was administered in the form of suspension with gum tragacanth, as compound was insoluble in water.

- Animals were fasted overnight.
In the morning drug was administered orally in stomach through rubber catheter. Animals were observed separately at 1 hr, 2 hrs and 4 hrs, mainly for their activity and if sleeping, score was noted; and at 24 hrs mortality was noted. The maximum possible dose of 2560 mg/100 g of the test compound can be administered in the suspension form. The observations were tabulated.

For Acute toxicity study in mice same procedure was carried out.

1.2 Subacute Toxicity Study

For this study, male albino adult rats weighing between 200 - 250 gms were selected and were grouped as in 7 groups one group was included as control and in each group there were six animals. Animals received following drugs daily orally

Control I Gum tragacanth 2ml
Group II Test compound 10 mg/100 gms
Group III Test compound 40 mg/100 gms
Group IV Test compound 160 mg/100 gms
Group V Test compound 640 mg/100 gms
Group VI Test compound 1280 mg/100 gms
Group VII Test compound 2560 mg/100 gms

Treatment was given over a period of 40 days. Every day mortality was noted and after 7 days weights were taken.

2) General behaviour study

For this study, male adult albino Rats were selected and grouped into 7 and one group was acting as control. Animals were treated with following drugs daily.

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Group II to Group VII test compound in the dose of 10, 40, 160, 640, 1280, 2560 mg/100gms.

Each animal was closely observed for its normal activity and sleeping score was given to each animal.

CNS stimulant or depressant activity was observed. Any changes in respiration or heart rate, was observed along with salivation.

3) Analgesic activity

1) Pinch Cock method

The pinch cock was applied about 2 cm from the base of the tail. By a preliminary screening Rats responding with continuous attempt to remove the pinch cock within 30 seconds were selected. Analgesic effect of the drug was considered present if the animal made no attempt to remove the pinch cock within 90 seconds.

This study was carried out in male albino rats weighing between 200 - 250 gms. The animals were screened before their inclusion in the study by applying pinch cock to the tail about 2 cm from base, and was marked by picric acid. Animals showing an attempt to remove pain producing stimuli i.e. attempting to remove pinch cock by mouth within 30 sec were selected to evaluate analgesic activity of test compound. Animals not showing any attempt to remove pinch cock within 30 sec. were excluded.

After initial screening of animals, they were grouped. Each group includes 6 Rats, which received following treatment. The night before animals were kept fasting.
Control I Gum tragacanth 2 ml
Group II Test compound 10 mg/100 gms
Group III Test compound 40 mg/100 gms
Group IV Test compound 160 mg/100 gms
Group V Test compound 640 mg/100 gms

Then at 2 hrs and 4 hrs analgesic activity i.e increased or decreased in pain threshold was noted in seconds.

3.2 Radiant heat method

An analgesio-meter (model Techno) was employed for this purpose. The Nicrome wire of the apparatus was heated with a current of 5 amp. Animals showing the "flicking" of the tail within 10 seconds, were chosen. Analgesic effect was considered to be present if no flicking occurred in 10 seconds.

Male Albino rats weighing between 200-250 gms were selected after screening. In rats pain (heat stimulus) was given by analgesiometer; by keeping tail of an animal 3-4 cm from from base on heating wire. Those animals showing tail flick within 10 sec of time were selected for further study of analgesic activity.

All selected Rats were grouped as follow and after overnight fasting next day morning they received following doses of test compound. In each group there were six animals, which were marked by picric acid for identification and weighed before drug administration. Test compound was freshly prepared in the form of suspension and administered orally.
ANTICONVULSANT ACTIVITY

ELECTRO CONVULSIOMETER
Analgesic activity, increased in pain threshold was assessed after 2 hrs and 4 hrs.

4) Antiepileptic activity

Antiepileptic efficacy was carried out in adult male albino rats weighing between 200 - 250 gms using following procedure.

4.1 Electroconvulsion method

The supramaximal shock, i.e. an electrical stimulus was given to each rat with the help of convulsometer. Ear clips (wet with saline) from the convulsimeter (Photograph) were applied to the ear of the rats. The strength of current was 150 mAmps. and duration was 0.2 sec. Rats exhibiting tonic extension of hind limbs followed by clonic convulsions were selected for estimation of antiepileptic activity.

Animals were kept fasting overnight on the day of experiment. Animals were grouped, and received following doses, orally in the form of suspension of test compound.

Control I 2 ml of gum tragacanth
Group II Test compound 10 mg/100 gms
Group III Test compound 40 mg/100 gms
Group IV Test compound 160 mg/100 gms
Group V Test compound 640 mg/100 gms
Three hours later each animal received electrical stimulus of the same strength and duration. The pattern of convulsion was observed in each animal. Abolition of tonic extensor phase was taken as a criteria of antiepileptic activity. Observations were tabulated.

4.2 Chemically induced convulsions

This study was carried out in male albino rats weighing between 200 - 250 gms. After marking, animals were grouped into 5 groups, each group having 6 animals. Animals were kept fasting overnight. On the day of experiment, animals received following doses of test compound orally.

Control I 2 ml gum tragacanth
Group II Test compound 10 mg/100 gms
Group III Test compound 40 mg/100 gms
Group IV Test compound 160 mg/100 gms
Group V Test compound 640 mg/100 gms

Three hours later, to each animal Pentylenetetrazole was injected intraperitoneally in a dose of 10mg/100 gms body weight, and the animal was observed for clonic convulsions.

The effects in each rat was noted and tabulated.
PENTOBARBITONE SLEEPING ACTIVITY

RAT – BEFORE & AFTER PENTOBARBITONE

LOSS OF RIGHTING REFLEX AFTER PENTOBARBITONE
5) Pentobarbitone sleeping time

Adult male albino rats weighing between 200 - 250 gms were used in this experiment. Animals were grouped into 5 groups. Each group included 6 animals and received following doses of test compound orally in the form of suspension.

<table>
<thead>
<tr>
<th>Group</th>
<th>Test compound</th>
<th>Dose (mg/100 gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Gum tragcanth</td>
<td>2 ml</td>
</tr>
<tr>
<td>II</td>
<td>Test compound</td>
<td>10</td>
</tr>
<tr>
<td>III</td>
<td>Test compound</td>
<td>40</td>
</tr>
<tr>
<td>IV</td>
<td>Test compound</td>
<td>160</td>
</tr>
<tr>
<td>V</td>
<td>Test compound</td>
<td>640</td>
</tr>
</tbody>
</table>

Three hours later to each animal pentobarbitone sodium was administered in dose of 3mg/100 gms intraperitoneally. For each animal following timings were recorded.

1) Time of administration.
2) Loss of Righting reflex
3) Regaining of Righting reflex
   \[2 - 1 = \text{onset of action}\]
   \[3 - 2 = \text{duration of sleep}\]

Observations were tabulated and analysed in all the groups.

6) Screening For Enzyme induction

This work was carried out in male albino rats weighing between 200 - 250 gms, to 50 animals. Phenobarbbitone sodium was
administered 6mg/100 gms body weight intraperitoneally, daily for 6 days prior to the experiment. On the day of experiment animals were grouped, in each group there were 6 animals and they received following doses of test compound.

Group I 2 ml of gum tragacanth
Group II 10 mg/100 gms test compound
Group III 40 mg/100 gms test compound
Group IV 160 mg/100 gms test compound
Group V 640 mg/100 gms test compound

(Group II - V: animals were pre-treated with phenobarbitone)

Three hours later, to each animal pentobarbitone sodium was administered intraperitoneally in a dose of 3 mg/100 g body weight and following observations were tabulated for each animal.

1) Time of administration
2) Time of Loss of Righting reflex
3) Time of Regaining of Righting reflex

2-1 = Onset of sleeping
3-1 = duration of sleep.

The effect of drug on the duration of sleeping time was analysed.
SWIMMING APPARATUS - TO EVALUATE SWIMMING ACTIVITY

Front View

Side View
7) Adaptogenic activity

This study was performed on adult male albino rats. Adaptogenic effect was measured by two methods.

7.1 Weight Gain

Rats were divided into following groups, and received different doses of test compound preparation orally in the form of suspension.

Control I 2 ml of Gum tragacanth
Group II Test compound 10 mg/100 gm
Group III Test compound 40 mg/100 gm
Group IV Test compound 160 mg/100 gm
Group V Test compound 640 mg/100 gm

Treatment was given over a period of 1 month and weights were measured on day 0, 7th day, 14th day, 21st day and 29th day and observations were tabulated.

7.2 Swimming Performance study

This study was carried out to evaluate the effect of drug (test compound) on swimming performance. It was studied by using the swimming apparatus (Photograph). Rat was released in the swimming apparatus. Rat starts swimming and to escape from the water it tries to climb over the wheel which leads to rotation of the wheel. These rotations of the wheel were recorded on attached counter. Rat continues to rotate till he gets exhausted and stops rotating the wheel. This entire performance was recorded.
After taking basal swimming performance count, the animals were grouped as follows. There were 6 animals in each group.

**Part I**

Control I Control 2 ml gum tragacanth  
Group II Test compound 10 mg/100 gms  
Group III Test compound 40 mg/100 gms  
Group IV Test compound 160 mg/100 gms  
Group V Test compound 640 mg/100 gms  

Above doses were administered for one month in the form of suspension orally by rubber catheter.

Swimming performance was recorded on day 0 as basal reading and after then on 7th, 14th, 21th and 28th day.

In part II of study lower doses of the test compound were tested as in part I  
Control I Control 2ml gum tragacanth  
Group II Test compound 5 mg/100 gms  
Group III Test compound 10 mg/100 gms  
Group IV Test compound 20 mg/100 gms  
Group V Test compound 40 mg/100 gms  

8) Extraction of test compound

Different extracts were prepared from the test compound in the powder form. 500 gms of powder was filled in a apparatus and serially extractions were carried out with petroleum ether 40 - 60, petroleum ether 60 - 80, solvent ether, chloroform separately. After extraction, the different solvents were evaporated to dryness in a water bath or under reduced pressure. The fractions obtained were tested for cardiovascular effect and effect on respiration.
In second extraction procedure, 500 gms of powder was soaked in 1:1 alcohol water mixture and after 2 days supernatent was taken, solvent was evaporated and dry residu e weighed and same dry residue was tested for its effect on blood pressure and respiration.

In all other studies, where the compound was given orally no extration was carried out, but the whole material was given in the form of suspension.

9) ATOMIC ABSORPTION SPECTROPHOTOMETRY

This study was carried out in vitro to analyse the percentage of different metal contents by using Atomic Absorption Spectrophotometer; Suvarnamakshikadiwati, an test compound was subjected to digestion, i.e. sample was processed.

A mixture of hydrochloric acid and nitric acid was used for dissolution. A suitable dissolution method was as follows.

One gm of sample, taken in porcilin dish. 10 ml of deionised water, 5 ml of hydrochloric acid and 5 ml of nitric acid added warmed on hot plate then kept in furnace at a temperature 500°C. 2 days, then before estimation, solution was filtered through Whatman No 42 paper. Cooled and transfered to 100 ml volumetric flask and diluted up to mark with water. This solution was used to identify an different elements, viz. Nickel, Copper, Cadmium, and Iron by using standard solutions of these elements for comparision and quantitation.

10) Hepatoprotective activity

Animals- adult male albino rats of Hafkin strain weighing 200-250 gms were used for study. Acute hepatocellularar damage was
induced by administration of carbon tetra chloride. Animals were
divided in 5 groups, each group consisted of 6 rats. All the
drugs were administered orally.

I  Control - 2 ml Gum tragacanth
II 0.2 ml CCl₄ - 0.2ml liquid paraffin
III 0.4 ml CCl₄ in liquid paraffin - 40 mg/100gm
     of test compound
IV 0.4 ml CCl₄ in liquid paraffin -160 mg/100gm of test
     compound
V 0.4 ml CCl₄ in liquid paraffin -640 mg/100gm of
    test compound

Test compound was administered in the form of
suspension, using gum tragacanth as suspending agent. Treatment was
given every day to overnight fasted rats, to ensure good and
consistent absorption of the drug. Rats were allowed food and
Glucose and salt water ad libidum, treatment was given for four
days.

Assessment of hepatoprotective activity

Susceptibility of the liver to damage by drugs is a
consequence of its primary role in drug metabolism. Hundreds of
compounds, both organic and inorganic are capable of causing
liver injury when they gain access to the body. Hepatoprotective
activity of various agents has been studied experimentally as
well as clinically. A majority of these agents have been studied
experimentally against chemically induced hepatic damage. Carbon
tetrachloride is the most common hepatotoxin used in these
studies.
On fifth day, dissection of animals was performed under ether anesthesia. Blood samples were drawn by cardia puncture for estimation of

1) Serum alkaline amino transferase (S-ALT)
2) Serum bilirubin
3) Serum alkaline phosphatase (S - ALP) by standard techniques. The animals were then sacrificed. The livers were removed, weights and volumes were noted. They were subjected to histopathological examination by a pathologist, who was kept unaware of the treatment received by the animals. Histopathological changes were noted.

11) Effect of suvarnamakshikadi-wati on Dog Blood Pressure and respiration.

5 healthy mongrel dogs of either sex weighing between 10 to 13 kg were used. They were anesthetised with pentobarbitone sodium 35 mg/kg I.V. Femoral blood pressure was recorded on kymograph by means of mercury manometer.

Trachea was cannulated and respiration was recorded through Mares' y tambour. The preparation was allowed to stabilise for 1/2 an hour. Drugs were injected through the cannulated left femoral vein. The normal response bracket consisting of saline, Adrenaline, Nor-adrenaline, Isoprenaline, acetylcholine and Histamine was recorded. Suvarnamakshikadi-wati 50% hydro alchoholic extract administered I.V. in dosage ranging from 1 ml. to 5 ml. and then after bracket responses were repeated, to see any change in response. In another set of experiment suvarnamakshikadi wati was administered orally ranging from 1 g to 4 gm. After half an hour, and
12) To study the effect of test compound on haemoglobin content.

This study was carried out as follows:

Animals: Healthy adult rats
No.: 40
Weight: 200 - 250 gms

Day 0: Basal haemoglobin levels were measured with a haemoglobinometer. All showed normal Hb levels.

During the period of 0-7 days anaemia was produced by bleeding animals every day for 7 days by tail-cutting and bleeding 2 ml of blood. On 8th day - Again haemoglobin content was measured.

Then animals were grouped as follows and received the treatments as indicated. In each group there were 10 animals.

Control I: Gum acacia 2 ml
Group II: Test compound 640 mg/100 gms

Treatment was given for 1 month and on 14th, 21st, 28th and 35th day again the haemoglobin contents were measured.