Final Report

EFFECT OF TRADITIONALLY USED AYURVEDIC RASA AUSHADHIES ON RENAL AND HEPATIC FUNCTIONS: CLINICAL AND EXPERIMENTAL STUDY

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(November 2009 – March 2014)

Submitted to: DEPARTMENT OF AYURVEDA, YOGA and NATUROPATHY, UNANI, SIDDHA AND HOMOEOPATHY (AYUSH), MINISTRY OF HEALTH and FAMILY WELFARE, GOVERNMENT OF INDIA
1. **Title of the study:** Effect of traditionally used Ayurvedic rasa aushadhis on renal and hepatic functions: clinical and experimental study.

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     Department of Pharmacology, AIIMS, New Delhi

5. **Non- scientific Staff engaged in the study:**
   - **Pankaj Sharma**, Department of Pharmacology, AIIMS, New Delhi

6. **Implementing Institution and other collaborating Institutions:**
   All India Institute of medical Sciences, Ansari Nagar, New Delhi – 110 029

7. **Date of commencement:** November 2009

8. **Duration:** 4 years & 4 months

9. **Date of completion:** March 2014
10. Objectives as approved:

1. To administer graded doses of Sidh Makardhwaj and Arogyavardhini Vati in rats for 4 weeks and evaluate hepatic and renal function tests and correlate with level of heavy metals in their blood.

2. To assess kidney and liver function tests of patients on treatment with (a) Sidh Makardhwaj for joint disorders (Rheumatoid arthritis) and (b) Arogyavardhini vati for dyslipidaemia.

3. To estimate level of mercury in blood and urine of patients, who are on treatment with the above ayurvedic rasa aushadhis and correlate their hepatic and renal function test parameters.

4. To estimate levels of urinary metallothionein, β-2 microglobulin and correlate with renal function tests and level of heavy metals in urine.

5. To evaluate the cytotoxic effect of Arogyavardhini vati, Kajjali, Ras sindur & Sidh Makardhwaj and on HepG2 and HEK cell using MTT assay.
11. Deviation made from original objectives if any, while implementing the project

And reasons thereof:

1. **Modification:** Minor modification has been done in the objective one.

   **Original Objective:** To administer graded doses of Sidh Makardhwaj and Arogyavardhini Vati in rats for 4 weeks and evaluate hepatic and renal function tests and correlate with level of heavy metals in their blood

   **Modified objective:** To administer graded doses of Sidh Makardhwaj and Arogyavardhini vati in rats for 4 weeks and evaluate cognitive function and oxidative stress, acetyl cholinesterase (AChE) activity and correlate with level of heavy metals.

2. **Addition:** The objective number five i.e. To evaluate the cytotoxic effect of Arogyavardhini vati, Kajjali, Ras sindur & Sidh Makardhwaj and on HepG2 and HEK cell using MTT assay, was also included

   **Reason:** In the review committee meeting, it was decided that in addition to renal and hepatic function, the effect on cognition, oxidative stress and acetyl cholinesterase (AChE) activity should also be done (Objective 1). The addition of the objective five was done because this additional data generation can be done within the allocated budget. Further this will be value addition to the project.
Experimental study
12. Experimental work giving full details of experimental set up, methods adopted, data collected supported by necessary tables, charts, diagrams and photographs.

A) Methodology followed for study of Sidh makardhwaj and Arogyavardhini vati in rats

i) Animals: Male Wistar rats (150-200 g) were used in the present study. The animals were obtained from the Central Animal Facility of All India Institute of Medical Sciences, New Delhi and stock bred in the departmental animal house. The rats were group housed in polyacrylic cages (38x23x10 cm) with not more than 3 animals per cage and maintained under standard laboratory conditions with natural dark and light cycle. They were allowed free access to standard dry rat diet (Ashirwad, Punjab, India) and tap water ad-libitum. However, 12 hrs before the behavioral testing, the rats were deprived of food as this is known to enhance their motivation to perform the test. All experimental procedures described were reviewed and approved by the Institutional Animal Ethics Committee, All India Institute of Medical Sciences, New Delhi India (497/IAEC/09).

ii) Drug treatment in rats:

1. Sidh Makardhwaj (Maharshi Ayurveda Pharmaceutical Limited, New Delhi, India) was suspended in honey (Dabur Pharmaceuticals Pvt. Ltd, Ghaziabad, India). Rats were randomly divided into four groups consisting of 6 rats each i.e., normal control, and Sidh Makardhwaj (10, 50 and 100 mg/kg) treated group. The lower dose of Sidh Makardhwaj in rat was calculated by extrapolating the equivalent human dose and was administered orally between 10 and 11 A.M. every day for 28 days, in a volume not exceeding 1 ml/100 g rat weight. Rats were weighed every 3rd day before administering the drug. On 29th day, the rats were subjected to the behavioural tests.
and then sacrificed for estimation of liver function tests (LFT), kidney function tests (KFT), malondialdehyde and glutathione, AChE activity, histopathological changes and mercury levels in rat’s liver, kidney and brain.

2. Arogyavardhini vati (procured from Maharshi Ayurveda Pharmaceutical Limited, New Delhi, India) was powdered and suspended in water. Rats were randomly divided into four groups consisting of 6 rats each i.e., normal control, and Arogyavardhini vati (50, 250 and 500 mg/kg) treated group. The lower dose of Arogyavardhini vati in rat was calculated by extrapolating the equivalent human dose and administered orally between 10 and 11 A.M. every day for 28 days, in a volume not exceeding 1 ml/100 g rat weight. Rats were weighed every 3rd day before administering the drug. On 29th day, the rats were subjected to the behavioral tests and sacrificed for estimation of liver function tests (LFT), kidney function tests (KFT), malondialdehyde and glutathione, AChE activity, histopathological changes and mercury levels in rat’s liver, kidney and brain.

iii) Neurobehavioral activity

a) One Trial Passive Avoidance Task: Memory retention deficit was evaluated by a step through passive avoidance apparatus (UgoBasile, USA) according to the method described by Nakahara et al, 1998. The apparatus consisted of 2 separate chambers connected through a guillotine door. One chamber was light using a bulb, while the other was dark (Fig 12.1). The floor of both the chambers consisted of steel grids, used to deliver electric shocks. Briefly, on the acquisition trial, each rat was placed in a lighted chamber. After 60 s of habituation, a guillotine door separating the light and dark chambers was opened, and the initial latency (IL) to enter the dark chamber was
recorded. Rats exhibiting an initial latency time of more than 60 s were excluded from further experiments. Immediately after the rat enters the dark chamber, the guillotine door was closed and an electric foot shock (75 V, 0.2mA, 50 Hz) was delivered to the floor grids for 3 s. The rat was removed from the dark chamber 5 s later and returned to its home cage. After 24 h, retention latency (RL) time was noted in the same way as in the acquisition trial, but foot shock was not being delivered, and the latency time was recorded up to a maximum of 600 s.

Figure 12.1: Photographs of apparatus used for neurobehavioral activities assessment
**b) Elevated plus maze:** The elevated plus maze consists of two closed arms and two open arms forming a cross, with a quadrangular center. The maze was placed above the floor. Acquisition and retention of memory processes was assessed using elevated plus maze according to the method described by Sharma and Kulkarni, 1992. On 1\textsuperscript{st} day, the 1\textsuperscript{st} trial (initial transfer latency) was carried out as follows: the rats were placed individually at the end of one open arm facing away from central platform and the time it took to move from the open arm to either of the enclosed arm (transfer latency) was recorded. Transfer latency was the time that elapsed between the times when its four legs crosses to the enclosed arm. In this experiment when the rat did not enter the enclosed arm for within 60 s, it was gently pushed on the back into the enclosed arm and the transfer latency was assigned 60 s. The rat was allowed to move freely in the plus maze regardless of open and closed arms for 10 s after the measurement of transfer latency. The rat was then gently taken out of the plus maze and was returned to its home cage. Twenty-four hours later, the 2\textsuperscript{nd} trial (retention transfer latency test) was formed. The rats were again put into the elevated plus maze. If the rat did not enter the enclosed arm within 60 s on 2\textsuperscript{nd} trial, the transfer latency was assigned 60s.

**c) Morris water maze:** The water maze consisted of a large circular pool (1.8 m in diameter, 0.6 m in height). A white platform (10 cm in diameter) was placed inside, and the tank was filled with water (22°C) until the top of the platform was submerged 1 cm below the water’s surface. In addition to the visual cues on the walls of the laboratory (shapes), five sheets of paper with black-and-white geometric designs attached to the sides of the tank served as additional cues. An automated tracking system (Video tracking system, Stoelting, USA) analyzed the swim path of each
subject and calculated escape latencies (the time between being placed in the water and finding the hidden platform), total path lengths, average swim speed, and thigmotaxia (percentage of time spent in periphery). Before beginning acquisition training rats were given a pre-training acclimation session during which they were allowed to swim in the pool for 5 min without the platform present. Beginning on the following day, rats were given seven acquisition sessions that consisted of four trials per day with an inter-trial interval of 10 min. Throughout the course of this acquisition period, the hidden platform remained in the same fixed position for all rats. Four points along the perimeter of the maze arbitrarily designated as N, S, E, and W, served as starting points where the rats were released, facing the wall of the tank, at the beginning of each trial (the order of the starting points was determined randomly, except that each starting point was used only once each session). Once a rat located the platform, it was allowed to remain there for 10 s before being removed from the tank. If a rat failed to locate the platform within 120 s, it was manually guided to it.

d) **Rota rod:** Rota rod was used to evaluate the muscle coordination of rats. Rats were conditioned to the accelerating rod (Ugo Basile). Each animal received training session on the rota rod at constant speed of 8 rpm and was tested until it achieves the criteria of remaining on the rotating spindle for 60 seconds. Each rat received single base line trial on the accelerating rota rod in which the spindle will increase from 4 to 40 rpm over a period of 5 minutes. After administration of selected drugs for 28 days, each rat received a test trial (Rogers et al., 1997).
iv) Biochemical Estimation and Histopathology

Following the behavioral testing, the animals were decapitated under ether anesthesia and the liver, kidney and brain were quickly removed. Half tissues were cleaned with ice cold saline and stored at –80° C and remaining were kept in 10% formalin for histopathological study.

**Biochemical Estimations**

*Oxidative stress parameters:* Liver, kidney and brain tissue samples were thawed and 10 % (w/v) homogenate was made with ice- cold 0.1 M phosphate buffer (pH 7.4). Aliquots of homogenates were used to determine MDA and GSH levels.

*Measurements of lipid peroxidation:* Malondialdehyde (indicator of lipid peroxidation) was estimated as described by Ohkawa et al, 1979. The reagents acetic acid 1.5 ml (20% v/v) pH 3.5, 1.5 ml thiobarbituric acid (0.8% w/v) and 0.2 ml sodium dodecyl sulphate (8.1% w/v) were added to 0.1 ml of processed tissue samples, and then heated at 95⁰C for 60 minutes. The mixture was cooled with tap water and 5 ml of n-butanol/pyridine (15:1), 1 ml of distilled water was added. The mixture was vortexed vigorously. Then samples were centrifuged at 4000 rpm for 10 min. The organic layer was separated and absorbance was measured at 532 nm using spectrophotometer. The concentration of MDA is expressed in nmol/g-wet tissue.

*Measurement of reduced glutathione:* Glutathione was measured according to the method of Ellman, 1959. Equal quantity of homogenate was mixed with 10% trichlororacetic acid and centrifuged to separate the proteins. To 0.1 ml of this supernatant, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5’5-dithiobis (2-nitrobenzoic
acid) and 0.4 ml of double distilled water were added. The mixture was vortex and the absorbance read at 412 nm within 15 min. The concentration of reduced glutathione was expressed as mg/g wet tissue.

**Acetyl cholinesterase (AChE) Activity:** AChE activity was done using the method of Ellman et al. (1961). Protein estimation was carried out by the method of Lowry et al. (1951).

![Double beam Ultraviolet spectrophotometer (SPECORDE 250)](image)

**Figure 12.2:** Double beam Ultraviolet spectrophotometer (SPECORDE 250) used for biochemical estimations

**Determination of kidney and liver function test:** To assess the state of the liver and kidney, serum urea and creatinine, SGOT, SGPT, ALP and bilirubin levels were estimated by using semi autoanalyzer (Mini techno, USA). Concentration of the enzymes was evaluated according to the instruction of manufacturer of assay kits (Logotech India Pvt. Ltd, Delhi, India).
Figure 12.3: Semi-auto analyzer (Mini techno, USA.) used for KFT and LFT estimations.

*Mercury estimation in liver, kidney and brain tissue:* Mercury was estimated in liver, kidney and brain tissue by inductively coupled plasma – atomic emission spectrophotometer (ICP-AES) and level was expressed in μg/g of wet brain tissue.

Figure 12.4: Photographs of ICP-AES used for heavy metal analysis in biological tissues.
**Histopathological study:** The tissues fixed in 10% formalin were processed through Xylol and ethanol and embedded in paraffin blocks as routine manner. Sections of 6µm thickness were cut and stained with Haematoxylin and Eosin (H and E) stain.

**Statistical analysis:** The data was expressed as Mean ± SEM (Standard Error of the Mean). Treated and control groups were compared using a one-way analysis of variance (ANOVA), followed by Posthoc multiple Comparisons of Tuckey Test. A 95% confidence level was used to determine statistically significant differences between treated and control groups. SPSS (version 16) for windows was applied for the analysis of data and p < 0.05 was taken as the level of significance.
Clinical study

(Clinical Trial Registry of India: REFCTRi- 2009 000699)
B) Methodology followed for evaluation of efficacy and safety of Ayurvedic treatments: a pilot prospective cohort clinical study

1. Ashwagandha powder and Sidh Makardhwaj in rheumatoid arthritis patients

Clinical study design

_Type of Study:_ Prospective, open-label, non-randomized, outpatient-based, single centered drug trial.

_Site of clinical study:_ CGHS Ayurvedic hospital, Ali Ganj, Lodhi Road, New Delhi

_Site of biochemical estimation:_ Department of Pharmacology, All India Institute of Medical Sciences, New Delhi.

_Inclusion Criteria_

a) Patient of either sex of age 18-60 years.

b) Patient diagnosed with joint disorder

c) Requires treatment with Sidh Makardhwaj for a period of 4 weeks or more.

d) Patient able to provide written informed consent.

_Exclusion Criteria_

a) Medical history of unstable angina, myocardial infarction, heart failure or stroke within 3 months of the study

b) Uncontrolled hypertension (diastolic blood pressure >100 mm Hg)

c) Uncontrolled diabetes mellitus.

d) ALT and AST > 2 x upper limit of normal (40mg/dl)

e) Impaired renal function (creatinine ≥2.0 mg/dl)

f) Pregnancy/Lactation

g) Patients on any Ayurvedic drugs during the last 15 days.
Clinical Trial Registry of India, Indian Council of Medical Research (CTRI, ICMR): The present study was registered with Clinical Trial Registry of India, Indian Council of Medical Research, New Delhi, India (Vide the approval number REFCTR-2009 000699)

Sample Size Determination: The sample size was determined by the following assumptions (a) There would be an 80% power for detecting a change from baseline in the Disease Activity Score in 28 joints (b) 20% improvement as per ACR criteria (ACR 20) response at fourth week (c) The test of the null hypothesis was conducted at a 2-sided 5% significance level. Under these assumptions, the required sample size was 75 subjects. However, considering a higher dropout rate and lack of sufficient data from published rheumatoid arthritis drug trials using Ayurvedic medicines, it was then decided to enrol 90 patients.

Patients enrolled: The first 125 patients were screened in OPD at CGHS Ayurvedic hospital. 86 patients satisfied the inclusion criteria and who were willing to participate in the study, signed the informed consent before enrolment. Detailed medical history, general physical examination and rheumatologic evaluation were recorded by the designated Ayurvedic physician and investigator of the trial. Laboratory tests were carried out as per protocol. Subsequently, all patients were examined by physician at every visit during the trial. At the end of the study, 78 patients completed and were adhered to study protocol. 8 patients withdrew from the study (2= due to unknown reason, 2= shifted to allopathic medication, 4= concomitant use of other medication).
Figure 12.5: Design of clinical study of Sidh Makardhwaj in rheumatoid arthritis patients

Medications: Patients took 5g of Ashwagandha choorna for first 21 days and 100 mg of Sidh Makardhwaj with honey daily for next 28 days. No concurrent analgesics / NSAIDs in any form, oral, injectable or topical, were permitted. Patients were not given any advice on lifestyle modification and diet.
**Samples collection:** From each patient 5 ml blood and 50 ml urine samples were taken at each visit. This sample was taken by a phlebotomist into plane tubes, centrifuged at 3,000g and stored at -80°C until biochemical analysis.

**Clinical assessment:** The primary efficacy end point was the proportion of patients with a 20% improvement as per ACR criteria (ACR20) response at fourth week. Secondary end points included ACR50 and ACR70 responses, change from baseline in the Disease Activity Score in 28 joints (DAS28), categorical analyses of DAS28/European League Against Rheumatism (EULAR) response, change from baseline in each of the ACR core set of parameters, changes in the Short Form 36 (SF-36).

The Disease Activity Score (DAS) is a combined index that has been developed in Nijmegen in the eighties to measure the disease activity in patients with Rheumatoid Arthritis (RA). It has been extensively validated for its use in clinical trials in combination with the EULAR response criteria. It is easy use makes it also possible to collect valuable information about the disease activity of patient in daily clinical practice. Evaluation of response to a treatment
can be made much easier and more objective using the DAS. Just assess the number of swollen and tender joints and measure the ESR. The DAS will provide a number between 0 and 10, indicating how active the rheumatoid arthritis is at this moment. Using the DAS, several thresholds have been developed for high disease activity, low disease activity or remission. Also response criteria have been developed based on the DAS, so when the DAS of a patient is measured at two time points (e.g. before the start of a treatment and after treatment), the patients clinical response can be assessed. Calculation of DAS28 is as follows.

\[
\text{DAS28} = 0.56\sqrt{\text{tender joints}} + 0.28\sqrt{\text{swollen joints}} + 0.70\ln(\text{ESR/CRP}) + 0.014\text{VAS}
\]

A DAS28 score of higher than 5.1 is indicative of high disease activity, whereas a DAS28 below 3.2 indicates low disease activity. A patient is considered to be in remission if they have a DAS28 lower than 2.6. Remission criteria are as follows.

**Table: 12.1: DAS criterion for assessment of improvement in patients with RA.**

<table>
<thead>
<tr>
<th>Present DAS 28</th>
<th>DAS 28 improvement over time points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt; 1.2</td>
</tr>
<tr>
<td>&lt; 3.2</td>
<td>Good response</td>
</tr>
<tr>
<td>3.2-5.1</td>
<td>Moderate response</td>
</tr>
<tr>
<td>&gt; 5.1</td>
<td>Moderate response</td>
</tr>
</tbody>
</table>

Safety assessments included hepatic function (ALT, AST, ALP and bilirubin) and renal function (Urea and creatinine) tests. Early sensitive marker of liver (β2 microglobulin) and kidney (NGAL: Neutrophil Gelatinase Associated Lipocalin) were assessed to evaluate the effect of the Sidh Makardhwaj exposure.
2. Arjuna powder and Arogyavardhini vati in dyslipidemia patients

Clinical study design

*Type of Study:* Prospective, open-label, non-randomized, outpatient-based, single centered drug trial.

*Site of clinical study:* CGHS Ayurvedic hospital, Ali Ganj, Lodhi Road, New Delhi.

*Site of biochemical estimation:* Department of Pharmacology, All India Institute of Medical Sciences, New Delhi.

*Inclusion Criteria*

a) Patient of either sex of age 18-60 years.

b) Plasma levels $\geq$200 mg/dL for TC, $\geq$130 mg/dL for LDL-C, $<40$ mg/dL for HDL-C, and $\geq$150 mg/dL (according to Adult Treatment Panel III, 2001)

c) Serum CRP levels $>5$ mg/dL.

d) Patient able to provide written informed consent.

*Exclusion Criteria*

h) Medical history of unstable angina, myocardial infarction, heart failure or stroke within 3 months of the study

i) Uncontrolled hypertension (diastolic blood pressure $>100$ mm Hg)

j) Uncontrolled diabetes mellitus.

k) ALT and AST $>2$ x upper limit of normal (40mg/dl)

l) Impaired renal function (creatinine $\geq2.0$ mg/dl)

m) Pregnancy/Lactation

n) Patients on any Ayurvedic drugs during the last 15 days.
Clinical Trial Registry of India, Indian Council of Medical Research (CTRI, ICMR): The present study was registered with Clinical Trial Registry of India, Indian Council of Medical Research, New Delhi, India (Vide the approval number REFCTR-II-2009 000699).

Sample Size Determination:
On the basis of the published literature, we anticipated reductions of at least 10% of total cholesterol, LDL, HDL and triglycerides at the end of treatment. Considering a higher dropout rate (20%) and lack of sufficient data from published hypolipidemias drug trials using ayurvedic medicines, we estimated that a sample size of 90 would provide at least 80% power to detect differences from baseline values, using a two-tailed α value of 0.05 and an estimated within-group SD of 10%.

Patients enrolled: The first 110 patients were screened in OPD at CGHS Ayurvedic hospital, New Delhi. 101 patients satisfied the inclusion criteria and willing to participate in the study, signed the informed consent. The reason for drop out was concomitant use of allopathic medications (8 of 14), non compliance to medication (3 of 14) and unknown reason (3 of 14). At the end of the study, 87 patients completed and were adhered to study protocol. 14 patients withdrew from the study (3= due to unknown reason, 3= non compliance to medication, 8= concomitant use of other medication).
**Figure 12.6: Design of clinical study of Arogyavardhini vati in dyslipidemia patients**

*Medications:* patients received Arjuna powder (5 g, twice a day) for the first 3 weeks followed by Arogyavardhini Vati (500 mg, twice a day) for 4 weeks. The study participants were not blinded to the study treatment during the entire week period. However, final data were reviewed independently blinded to the investigators.
**Samples collection:** Five milliliter blood was collected by a phlebotomist into plane tubes from all patients on day of enrollment and at the end of the study. Fresh, clear, unhemolyzed serum was collected as the specimen with the patient fasting for 12 hours prior to specimen collection. Samples were centrifuged at 3000 rpm and serum was stored at -80°C for biochemical analysis. 50 ml urine samples were collected similarly in a sterile sample container to estimate the level of mercury in urine by ICP-AES, 2000-2, Jobin Horiba, France.

**Clinical assessment:** Lipid lowering effect of Arogyavardhini Vati was measured by comparing the lipid profile levels tested on initiation of the study (day 1) to the end of the study. Efficacy was measured by percent reduction in lipid profile levels as compared to baseline. Serum total cholesterol, triglyceride, LDL, HDL, LFT and KFT were analyzed by semiauto analyzer (Mini Techno, USA). Safety assessments included hepatic function (ALT, AST, alkaline phosphatase (ALP), and bilirubin) and renal functions (urea and creatinine) tests. An early sensitive marker of liver (β2 microglobulin) and kidney (NGAL: neutrophil gelatinase-associated lipocalin) were assessed to evaluate the effect of the Arogyavardhini Vati exposure. LFT and KFT levels were correlated with the urine mercury level. Serum total cholesterol, triglyceride, LDL, HDL, LFT and KFT were analyzed by a semi auto analyzer (Mini Techno, USA).
Cell Culture study
C) Methodology followed for Cell culture study

i) Drugs, Chemicals and Growth Medium

Mercury (Merck, USA), gold (SRL, India) and (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide ((MTT), (Sigma, USA)) were purchased. Dulbecco’s Modified Eagle’s Minimal Essential Medium (DMEM), Penicillin-Streptomycin and fetal bovine serum (FBS) were purchased from Hi- Media, USA. Sidh Makardhwaj and Arogyavardhini vati, Rasa sindur and Kajjali were purchased from good manufacturing practice (GMP) certified company (Maharishi Ayurveda Products Pvt. Ltd., India).

ii) Cell Cultures

*Rationale for cell line selection:* Mercury is a well known toxic heavy metal and major target organs are liver and kidney. Therefore, HepG2 (liver) and HEK (kidney) cell line were used in cell culture study.

HepG2 and HEK cells lines were obtained from National Centre for Cell Sciences (Pune, India). Cells were grown in 75cm² tissue culture flasks, diluted with DMEM supplemented with 10% fetal bovine serum (FBS) and 1% streptomycin and penicillin and incubated for 24 hours at 37°C in a 5% CO₂ incubator. Cells were grown to 80-95% confluence. Cells were then washed with phosphate buffer saline (PBS) and trypsinized with 1ml of 0.25% (w/v) trypsin, 0.03% (w/v) ethylenediaminetetraacetate (EDTA). Cell were diluted with above solution, counted and seeded (5 x 10⁵ cells/well) in two sets of 96 well micro titer tissue culture plates. The resulting cell suspension was used for experiment (Tarabová et al., 2006; Amara et al., 2010; Arnal et al., 2012).
iii) Positive control

*Rationale for positive control selection:* Sidh Makardhwaj is a popular Kupipakwa rasayan, prepared with swarna (gold), parada (mercury), gandhaka (sulphur) in a specific ratio mentioned in Ayurvedic Formulary of India (AFI, 2005). It is prepared by a specific process of constant heating for more than 24 hours and converting them in a stable compound. Arogyavardhini vati is a mercury based formulation mentioned in Ayurvedic Formulary of India (AFI, 2005). Therefore, mercury, gold, mixture of mercury and gold (Hg +Au) was selected as positive control.

Sulphur is known to be of low toxicity and poses very little if any risk to human and animal health. Oral LD$_{50}$ dose in rat is 5g/kg. The concentration of sulphur content in these Ayurvedic formulations are approximately 1000 times less than LD$_{50}$ dose. Hence, sulphur was not taken in positive control.

*Kajjali* is the black powder obtained from rubbing of mercury with sulphur. In general for preparation of *Kajjali*, mercury and sulphur are taken in a *Khalwa* and rubbed slowly till the mixture becomes black fine powder. Normally it takes 40 to 60 hours in 4 to 10 days for preparation of a good quality of *Kajjali*. Since, *Kajjali* contain mercury and sulphur, it will act as comparator of mercury alone effect and will demonstrate that Ayurvedic processing plays an important role in detoxification process and retaining therapeutic potential.

*Ras-Sindur* is freshly prepared in Damru-Yantra to obtain Parad. The obtained Parad is again mixed with Gandhak and Navsagar to obtain Kajjali which is cooked in Valuka-Yantra to get Ras-Sindur. Ras sindor was used as comparator of Sidh Makardhwaj.
iv) Dose calculation

*Rationale for dose selection:* Dose selections of drugs were based on the two presumptions (a) 100% bioavailability (b) cells are exposed to 100% drug. Dose of Sidh Makardhwaj, Arogyavardhini vati, Ras Sindur and Kajjali was equivalent to dose of mercury, gold, mixture of mercury and gold. The doses of drugs are selected upto 8 times of therapeutic dose (X, 2X, 4X, 8X).

*Rationale for exposure time selection:* Eukaryotic cell cycle is illustrated by human cells in culture, which divide approximately every 24 hours. The division cycle of most eukaryotic cells is divided into four discrete phases: M, G$_1$, S, and G$_2$. M phase (mitosis) is usually followed by cytokinesis. S phase is the period during which DNA replication occurs. Therefore, taking into consideration of all phases of cell cycle, drug exposure duration was taken as 3, 6, 12 and 24 hrs.

Mercury, gold, mixture of gold and mercury solution were used as positive control. The doses used in the present study were 1.65, 3.12, 6.25, 12.5, 25, 50 and 100 µg/ml and incubation duration was 3, 6, 12 and 24 hours.
a) Sidh Makardhwaj

Sidh Makardhwaj dose was calculated based on following three pre-assumptions

1. 100 mg of Sidh Makardhwaj dose is recommended for 60 Kg healthy human (average body weight) (Ayurvedic Formulary of India, 2005).

   100 mg of Sidh Makardhwaj for 5 L blood (Approximately 5L blood is present in healthy human being of weight 60 kg).

2. There is 100% bioavailability of Sidh Makardhwaj

   Hence, 100 mg Sidh Makardhwaj in 5L of blood (All the drugs taken orally by a healthy human being reaches in blood)

   \[ \approx 100,000 \mu g \text{ in } 5000 \text{ ml of blood} \]

   \[ \approx 20 \mu g/ml \]

3. 100% of Sidh Makardhwaj reaches to cells from blood

   Concentration of Sidh Makardhwaj in cell = 20 µg/ml

Sidh Makardhwaj is mixture of gold, mercury and sulphur in the ratio of 1:8:24 (Ayurvedic Formulary of India, 2005). Hence, mercury, gold and sulphur content in Sidh Makardhwaj at the doses of 20, 40, 80 and 200 µg/ml (X, 2X, 4X and 8X) will be as follows

<table>
<thead>
<tr>
<th>Sidh Makardhwaj dose</th>
<th>Gold</th>
<th>Mercury</th>
<th>Sulphur</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 µg/ml</td>
<td>0.6 µg</td>
<td>4.9 µg</td>
<td>14.5 µg</td>
</tr>
<tr>
<td>40 µg/ml</td>
<td>1.2 µg</td>
<td>9.8 µg</td>
<td>29.0 µg</td>
</tr>
<tr>
<td>80 µg/ml</td>
<td>2.4 µg</td>
<td>19.6 µg</td>
<td>58.0 µg</td>
</tr>
<tr>
<td>200 µg/ml</td>
<td>6 µg</td>
<td>48.5 µg</td>
<td>145.5 µg</td>
</tr>
</tbody>
</table>
b) Arogyavardhini vati

1. 500 mg of Arogyavardhini vati dose is recommended for 60 Kg healthy human (average body weight) (Ayurvedic Formulary of India, 2005).

500 mg of Arogyavardhini vati for 5 L blood (Approximately 5L blood is present in healthy human being of weight 60 kg).

2. There is 100% bioavailability of Arogyavardhini vati

Hence, 500 mg Arogyavardhini vati in 5L of blood (All the drugs taken orally by a healthy human being reaches in blood)

\[ \approx 500,000 \, \mu g \, \text{in} \, 5000 \, \text{ml of blood} \]

\[ \approx 100 \, \mu g/\text{ml} \]

3. 100% of Arogyavardhini vati reaches to cells from blood

Concentration of Arogyavardhini vati in cell = 100 µg/ml

Arogyavardhini vati is a polyherbal formulation (44 parts) and mercury is one ingredient (1 part) (Ayurvedic Formulary of India, 2005).

Hence, mercury content in Arogyavardhini vati at the doses of 100, 200, 400 and 800 µg/ml (X, 2X, 4X and 8X) will be as follows

<table>
<thead>
<tr>
<th>Arogyavardhini vati dose</th>
<th>Mercury</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µg/ml</td>
<td>2.8 µg</td>
</tr>
<tr>
<td>200 µg/ml</td>
<td>5.6 µg</td>
</tr>
<tr>
<td>400 µg/ml</td>
<td>11.2 µg</td>
</tr>
<tr>
<td>800 µg/ml</td>
<td>22.4 µg</td>
</tr>
</tbody>
</table>
c) Ras Sindur

Ras Sindur dose was calculated based on following three pre-assumptions

1. 125 mg of Ras Sindur dose is recommended for 60 Kg healthy human (average body weight) (Ayurvedic Formulary of India, 2005).

   125 mg of Ras Sindur for 5 L blood (Approximately 5L blood is present in healthy human being of weight 60 kg).

2. There is 100% bioavailability of Ras Sindur

   Hence, 125 mg Ras Sindur in 5L of blood (All the drugs taken orally by a healthy human being reaches in blood)

   \[ \approx 125,000 \, \mu g \text{ in } 5000 \, ml \text{ of blood} \]

   \[ \approx 25 \, \mu g/ml \]

3. 100% of Ras Sindur reaches to cells from blood

   Concentration of Ras Sindur in cell = 25 \, \mu g/ml

Ras Sindur is mixture of mercury and sulphur in the ratio of 1:6 (Ayurvedic Formulary of India, 2005).

Hence, mercury and sulphur content in Ras Sindur at the doses of 25, 500, 100 and 200 \, \mu g/ml (X, 2X, 4X and 8 X) will be as follows

**Table 12.4: Mercury and sulphur content in Ras Sindur (25, 500, 100, 200 \, \mu g/ml)**

<table>
<thead>
<tr>
<th>Ras Sindur dose</th>
<th>Mercury</th>
<th>Sulphur</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 , \mu g/ml</td>
<td>3.6 , \mu g</td>
<td>21.4 , \mu g</td>
</tr>
<tr>
<td>50 , \mu g/ml</td>
<td>7.2 , \mu g</td>
<td>42.8 , \mu g</td>
</tr>
<tr>
<td>100 , \mu g/ml</td>
<td>14.4 , \mu g</td>
<td>85.6 , \mu g</td>
</tr>
<tr>
<td>200 , \mu g/ml</td>
<td>28.8 , \mu g</td>
<td>171.2 , \mu g</td>
</tr>
</tbody>
</table>
d) Kajjali

Kajjali dose was calculated based on following three pre-assumptions

1. 250 mg of Kajjali dose is required for 60 Kg healthy human (average body weight) (Ayurvedic Formulary of India, 2005).

   250 mg of Kajjali for 5 L blood (Approximately 5L blood is present in healthy human being of weight 60 kg).

2. There is 100% bioavailability of Kajjali
   
   Hence, 250 mg Kajjali in 5L of blood (All the drugs taken orally by a healthy human being reaches in blood)
   
   \[ \approx 250,000 \mu g \text{ in } 5000 \text{ ml of blood} \]
   
   \[ \approx 50 \mu g/ml \]

3. 100% of Kajjali reaches to cells from blood
   
   Concentration of Kajjali in cell = 50 µg/ml

Kajjali is mixture of mercury and sulphur in the ratio of 1:6 (Ayurvedic Formulary of India, 2005).

Hence, mercury and sulphur content in Kajjali at the doses of 50, 100, 200 and 400 µg/ml (X, 2X, 4X and 8 X) will be as follows

**Table 12.5: Mercury and sulphur content in Kajjali (50, 100, 200, 400 µg/ml)**

<table>
<thead>
<tr>
<th>Kajjali dose</th>
<th>Mercury</th>
<th>Sulphur</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>7.2</td>
<td>42.8</td>
</tr>
<tr>
<td>100</td>
<td>14.4</td>
<td>85.6</td>
</tr>
<tr>
<td>200</td>
<td>28.8</td>
<td>171.2</td>
</tr>
<tr>
<td>400</td>
<td>57.6</td>
<td>342.4</td>
</tr>
</tbody>
</table>
Cell line exposures:

HepG2 and HEK cell lines were exposed to mercury, gold, mixture of mercury and gold at varying concentration (1.65, 3.12, 6.25, 12.5, 25, 50 and 100 µg/ml), Sidh Makardhvaj (20, 40, 80 and 200 µg/ml), Arogyavardhini vati (100, 200, 400 and 800 µg/ml), Ras Sindur (25, 50, 100 and 200 µg/ml) and kajjali (50, 100, 200 and 400 µg/ml) for 2, 6, 12, 24 hrs and following are the summary results of cell culture study

The varying concentration of mercury, gold, mixture of gold and mercury solution was used as positive control. Sidh Makardhwaj, Kajjali and Ras Sindur were dissolved in dimethy sulfoxide (DMSO). Arogyavardhini vati was made powder and suspended in normal saline.

The detailed dose calculation of Sidh Makardhwaj, Arogyavardhini vati, Kajjali and Ras Sindur for study were described in earlier tables (Table 12.2, 12.3, 12.4 and 12.5).

Cell viability assay:

The varying concentrations of mercury, gold, gold and mercury solution, Sidh Makardhwaj, Arogyavardhini vati, Kajjali and Ras Sindur were taken and incubation was done for 3, 6, 12 and 24 hours. After incubation and treatment, cells were treated with 10 µL of MTT (final concentration, 5 mg/ ml) for 4 hours. Dark blue formazan crystals were formed in intact cells. The supernatant was then removed. The formazan crystals were solubilized by adding 100 µL of anhydrous DMSO in each well. The extent of MTT reduction was measured using an ELISA reader (BioRad, Microplate reader, Model: 680, USA) at 450 nm with a 630 nm reference filter. Experiments were performed three times to obtain final percent cell viability.
Figure 12.7: MTT assay technique. A= 96 well microtitre culture plate, B= Exposure of cells with drugs and removing the media, C= MTT addition, D= Reading by ELISA reader.
Statistical analysis

The data was expressed as mean ± SEM (Standard Error of the Mean). A one-way analysis of variance (ANOVA), followed by Post hoc multiple comparisons of Tukey test was used for statistical analysis. SPSS (version 16) statistical software was used for the analysis of data and $p < 0.05$ was taken as the level of significance.
Effect of Sidh Makardhwaj on rat’s brain, kidney and liver
12.1: Effect of Sidh Makardhwaj on rat’s brain

**Effect of Sidh Makardhwaj on passive avoidance task (step-through paradigm):** The mean initial latencies of normal control, Sidh Makardhwaj of doses 10, 50 and 100 mg/kg were $13.9 \pm 3.2$, $8.5 \pm 2.4$, $10.9 \pm 3.2$ and $8.4 \pm 1.7$ s respectively and mean retention latencies on day 2\textsuperscript{nd} were $31.5 \pm 4.8$, $22.1 \pm 4.3$, $29.7 \pm 3.4$ and $23.3 \pm 4.3$ s, on day 7\textsuperscript{th} were $102.1 \pm 9.7$, $65.9 \pm 14.3$, $93.4 \pm 16.1$ and $100.2 \pm 21.1$ s, on day 14\textsuperscript{th} were $144.3 \pm 33.4$, $142.6 \pm 32.8$, $153.6 \pm 25.1$ and $175.8 \pm 19.3$ s and on day 28\textsuperscript{th} were $206.9 \pm 28.1$, $248.5 \pm 29.4$, $250.3 \pm 16.4$ and $238.6 \pm 21.1$ s. The results indicate that there were no significant differences among the mean retention latencies of all treated groups on day 2, 7, 14 and 28 as compared to normal group (Fig 12.1.1).

![Graph showing effect of Sidh Makardhwaj on step through latency in rat.](image)

**Figure 12.1.1:** Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on step through latency in rat.

**Effect of Sidh Makardhwaj on elevated plus maze:** The mean initial transfer latencies of normal control, Sidh Makardhwaj of doses 10, 50 and 100 mg/kg were $41.1 \pm 4.3$, $38.2 \pm 4.5$, $37.0 \pm 5.1$ and $38.6 \pm 4.4$ s respectively and mean retention transfer latencies on day 2\textsuperscript{nd} were $10.3 \pm 2.2$, $19.7 \pm 5.9$, $18.0 \pm 5.9$ and $10.4 \pm 1.1$ s, on day 7\textsuperscript{th} were $10.0 \pm 2.4$, $13.3 \pm 2.6$, $8.3 \pm 1.6$ and $6.8 \pm 1.3$ s, on day 14\textsuperscript{th} were $8.1 \pm 0.6$, $9.5 \pm 1.8$, $6.7 \pm 0.7$ and $6.3 \pm 0.8$ s and on day 28\textsuperscript{th} were $6.7 \pm 1.0$, $6.8 \pm 0.6$, $5.03 \pm 0.4$ and $4.4 \pm 0.4$ s. No significant
difference was found among the mean retention latencies of all treated groups on day 2, 7, 14 and 28 as compared to normal group (Fig 12.1.2).

![Graph showing mean retention latencies of all treated groups on day 2, 7, 14, and 28 compared to normal group.](image)

**Figure 12.1.2: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) elevated plus maze in rat**

*Effect of Sidh Makardhwaj on Morris water maze:* The mean distance traveled to reach platform by normal control, Sidh Makardhwaj treated group of doses 10, 50 and 100 mg/kg during the acquisition trial were 17.1 ± 4.5, 10.3 ± 0.8, 10.2 ± 1.4 and 10.4 ± 2.2 m, whereas during the probe trial were 6.0 ± 1.8, 4.9 ± 1.0, 4.8 ± 0.8 and 6.5 ± 1.1 m, on day 7th were 5.8 ± 1.6, 3.1 ± 0.6, 3.8 ± 0.5 and 3.0 ± 0.5 m, on day 14th were 4.7 ± 1.1, 2.1 ± 0.2, 2.9 ± 0.4 and 2.4 ± 0.2 m and on day 28th were 3.7 ± 0.6, 1.6 ± 0.2, 1.6 ± 0.4 and 1.6 ± 0.2 m. No significant difference was observed in the mean distance traveled to reach platform among the Sidh Makardhwaj treated groups as compared to normal group on day 0, 7, 14 and 28 (Fig 12.1.3, 12.1.4).

![Graph showing mean distance traveled to reach platform in Morris water Maze.](image)

**Figure 12.1.3: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on distance to travel to reach platform in Morris water Maze in rat**
Figure 12.1.4: Effect of Siddha Makardhwaj (10, 50 & 100 mg/kg) on Morris water Maze in rat (path track) 14: At day 0 (baseline), 15: At day 28 (After treatment).
**Effect of Sidh Makardhwaj on rota rod:** The mean retention time of normal group, Sidh Makardhwaj of doses 10, 50 and 100 mg/kg on day 1st were 171.3 ± 21.1, 166.6 ± 12.9, 158.3 ± 13.5 and 148.3 ± 16.7 s, on day 7th were 186.8 ± 15.9, 178.6 ± 11.4, 177.0 ± 11.7 and 184.5 ± 17.5 s, on day 14th were 187.6 ± 12.3, 187.5 ± 13.8, 201.0 ± 15.5 and 201.1 ± 15.1 s and on day 28th were 194.1 ± 13.6, 203.6 ± 9.8, 236.8 ± 16.2 and 225.8 ± 16.0 s. There were significant increased (p< 0.05) retention time on day 7th, 14th and 28th of normal control group and Sidh Makardhwaj treated groups as compared to day 1st retention time. Sidh Makardhwaj (10, 50 and 100 mg/kg, p.o) treatment for 28 days did not produced any significant decrease in the mean retention time ensuing restoration of motor coordination (Fig 12.1.5).

![Figure 12.1.5: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rota rod in rat](image)

**Effect of Sidh Makardhwaj on rat brain MDA level:** The rat’s brain MDA levels of normal control and 3 doses of Sidh Makardhwaj (10, 50 and 100 mg/kg) were 84.6 ± 4.3, 82.1 ± 3.2, 83.9 ± 5.2, 84.2 ± 3.7 nmol/g-wet tissue respectively. The results of the present study shows that there were no significant difference in MDA level of Sidh Makardhwaj (10, 50 and 100 mg/kg) treated groups as compared to normal group, shows no oxidative stress in Sidh Makardhwaj treated group (Fig. 12.1.6)
Effect of Sidh Makardhwaj on rat brain GSH level: The rat’s brain GSH levels of normal control, and 3 doses of Sidh Makardhwaj (10, 50 and 100 mg/kg) were 2.23 ± 0.06, 2.24 ±0.03, 2.25 ± 0.02, 2.27 ± 0.02 mg/g-wet tissue respectively. The results of the present study shows that there were no significant difference in GSH level of Sidh Makardhwaj (10, 50 and 100 mg/kg) treated groups as compared to normal group shows no oxidative stress in Sidh Makardhwaj treated group (Fig. 12.1.7).
**Effect of Sidh Makardhwaj on AChE activity of rat frontal cortex and hippocampus:** The rat’s frontal cortex and hippocampus AChE activity of normal control, and 3 doses of Sidh Makardhwaj (10, 50 and 100 mg/kg) were 24.94 ± 0.74, 23.83 ± 0.53, 23.70 ± 0.46, 23.21 ± 0.45 µM / g protein / min. and 29.36 ± 0.69, 28.88 ± 0.81, 27.94 ± 0.88, 27.81 ± 1.08 µM / g protein / min. respectively. The results of the present study shows that there were no significant difference in AChE activity of Sidh Makardhwaj (10, 50 and 100 mg/kg) treated groups as compared to normal group in frontal cortex (p= 0.89, p= 0.77, p= 0.62) as well as in hippocampus (p= 0.99, p= 0.85, p= 0.81) (Fig. 12.1.8, 12.1.9).

![Figure 12.1.8](image1)
**Figure 12.1.8:** Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rat’s brain AChE activity in frontal cortex

![Figure 12.1.9](image2)
**Figure 12.1.9:** Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rat’s brain AChE activity in hippocampus
Effect of Sidh Makardhwaj on brain mercury level: The rat’s brain mercury levels of normal control and 3 doses of Sidh Makardhwaj (10, 50 and 100 mg/kg) were 1.25 ± 0.32, 37.32 ± 3.34, 39.05 ± 1.35, 42.91 ± 4.54 µg/g-wet tissue respectively. The results of the present study shows significant increase in rat’s brain mercury levels of Sidh Makardhwaj (10, 50 and 100 mg/kg) treated groups (p= 0.024, p= 0.004, p< 0.001) as compared to normal group (Fig 12.1.10).

![Graph showing mercury levels](image)

Figure 12.1.10: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rat’s brain mercury level

Effect of Sidh Makardhwaj on brain histology: The brain of normal control showed no histopathological changes but the brain of rats treated with high dose of Sidh Makardhawaj (100mg/kg) was macroscopically slightly congested and microscopically brain sections revealed congestion of blood vessels, necrosis of Purkinje cells of the cerebellum, necroses of pyramidal cells. The histopathology of present study shows neurodegeneration at higher doses of Sidh Makardhawaj but not at therapeutic dose and duration (Fig 12.1.11).

![Histology images](image)

Figure 12.1.11: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rat’s brain histology
12.2: Effect of Sidh Makardhwaj on kidney in rats

*Effect of chronic administration of Sidh Makardhwaj on serum urea nitrogen:* Serum urea nitrogen level of normal control and Sidh Makardhwaj in the doses of 10, 50, 100 mg/kg treated groups were $14.15 \pm 1.92$, $15.63 \pm 4.78$, $17.93 \pm 3.93$, $19.20 \pm 3.96$ mg/dl, respectively. As compared the normal control group, there was no significant difference in the serum urea nitrogen levels of Sidh Makardhwaj treated group at doses of 10, 50 and 100 mg/kg, however, the serum urea nitrogen level was at the upper range of normal level in higher doses of Sidh Makardhwaj(100 mg/kg) treated group (Fig 12.2.1).

![Figure 12.2.1: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rat's serum urea nitrogen level](image)

*Effect of chronic administration of Sidh Makardhwaj on serum creatinine:* Serum creatinine levels of normal control and Sidh Makardhwaj in the doses of 10, 50, 100 mg/kg treated groups were $0.39 \pm 0.07$, $0.40 \pm 0.07$, $0.47 \pm 0.04$, $0.54 \pm 0.17$ mg/dl, respectively. As compared the normal control group, there was no significant difference in the serum creatinine levels of Sidh Makardhwaj treated group at doses of 10, 50 and 100 mg/kg, however, the serum creatinine level was at the upper range of normal level in higher doses of Sidh Makardhwaj(100 mg/kg) treated group (Fig 12.2.2).
Effect of chronic administration of Sidh Makardhawaj on kidney MDA levels: Kidney MDA levels of normal control and Sidh Makardhawaj in the doses of 10, 50, 100 mg/kg treated groups were 180.29 ± 3.48, 181.72 ± 1.64, 188.89 ± 4.42, 216.76 ± 20.54 nmol/g tissue, respectively. A significant increase in kidney MDA levels at higher dose of Sidh Makardhawaj (100 mg/kg) treated group (P < 0.05) was observed while there was no significant change in kidney MDA levels of Sidh Makardhawaj treated groups at lower doses (10 and 50 mg/kg) as compared to the normal control group (Fig 12.2.3).

Figure 12.2.2: Effect of Sidh Makardhawaj (10, 50 & 100 mg/kg) on rat’s serum creatinine level

Figure 12.2.3: Effect of Sidh Makardhawaj (10, 50 & 100 mg/kg) on rat’s kidney MDA level
Effect of chronic administration of Sidh Makardhawaj on kidney GSH levels: GSH levels in kidney tissue of normal control and Sidh Makardhawaj in the doses of 10, 50, 100 mg/kg treated groups were 4.02 ± 0.09, 3.96 ± 0.08, 3.66 ± 0.15, 2.57 ± 0.23 mg/g tissue, respectively. A statistically significant decrease in kidney GSH levels at higher dose of Sidh Makardhawaj (100 mg/kg) treated group (P < 0.05) was observed while there was no significant difference in kidney GSH levels at lower doses Sidh Makardhawaj treated group at dose of 10 and 50 mg/kg as compared to normal control group (Fig 12.2.4).

![GSH Levels Chart]

Figure 12.2.4: Effect of Sidh Makardhawaj (10, 50, 100 mg/kg) on rat’s kidney GSH level

Effect of chronic administration of Sidh Makardhawaj on mercury levels in kidney tissue: Mercury level in kidney tissues of normal control and Sidh Makardhawaj in the doses of 10, 50, 100 mg/kg treated groups were 115.94 ± 4.95, 181.91 ± 4.39, 377.04 ± 13.03, 500.01 ± 32.94 µg/g wet tissue, respectively. There was a significant increase in kidney mercury levels in higher doses Sidh Makardhawaj (50 and 100 mg/kg) treated groups (p< 0.001) as compared to normal control group. However, no statistically significant increase in kidney mercury level was observed with the lower dose of Sidh Makardhawaj (10 mg/kg) as compared to the normal control group (Fig 12.2.5).
Figure 12.2.5: Effect of Sidh Makardhwaj (10, 50, 100 mg/kg) on rat’s kidney mercury level

**Effect of Sidh Makardhwaj on kidney histology:** The kidneys of normal control showed no abnormal histopathological changes but the kidney of rats treated with higher dose of Sidh Makardhwaj (100 mg/kg) showed macroscopically slight congestion of blood vessels. Microscopically, kidney sections revealed congestion of blood vessels and epithelium disruption in proximal convoluted tubules at higher dose of Sidh Makardhwaj (100 mg/kg). The results of histopathology showed nephrotoxicity only at the higher doses of Sidh Makardhwaj (100 mg/kg) but not at therapeutic dose (Fig 12.2.6).

Figure 12.2.6: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rat’s kidney histology
12.3: Effect of Sidh Makardhwaj on liver in rats

Effect of chronic administration of Sidh Makardhwaj on serum ALT: Serum ALT level of normal control and Sidh Makardhwaj in the doses of 10, 50, 100 mg/kg treated groups were 129.48±1.39, 127.75±1.82, 128.83±2.85, 130.10±1.56 IU/l, respectively. As compared the normal control group, there was no significant difference in the serum ALT levels of Sidh Makardhwaj treated group at doses of 10, 50 and 100 mg/kg (Fig 12.3.1).

![Figure 12.3.1: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rat’s serum ALT level](image)

Effect of chronic administration of Sidh Makardhwaj on serum AST: Serum AST level of normal control and Sidh Makardhwaj in the doses of 10, 50, 100 mg/kg treated groups were 106.50±1.74, 109.12±1.64, 110.38±2.42, 109.42±1.95 IU/l, respectively. As compared the normal control group, there was no significant difference in the serum AST levels of Sidh Makardhwaj treated group at doses of 10, 50 and 100 mg/kg (Fig 12.3.2).
Figure 12.3.2: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rat’s serum AST level

Effect of chronic administration of Sidh Makardhwaj on serum ALP: Serum ALP level of normal control and Sidh Makardhwaj in the doses of 10, 50, 100 mg/kg treated groups were 41.92±0.53, 44.10±1.56, 45.37±1.66, 44.08±1.45 IU/l, respectively. As compared the normal control group, there was no significant difference in the serum ALP levels of Sidh Makardhwaj treated group at doses of 10, 50 and 100 mg/kg (Fig 12.3.3).

Figure 12.3.3: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rat’s serum ALP level
**Effect of chronic administration of Sidh Makardhwaj on serum bilirubin:** Serum bilirubin level of normal control and Sidh Makardhwaj in the doses of 10, 50, 100 mg/kg treated groups were 0.13±0.11, 0.16±0.02, 0.18±0.01, 0.17±0.01 mg/dl, respectively. As compared to the normal control group, there was no significant difference in the serum bilirubin levels of Sidh Makardhwaj treated group at doses of 10, 50 and 100 mg/kg (Fig 12.3.4).

![Figure 12.3.4: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rat's serum bilirubin level](image)

**Effect of chronic administration of Sidh Makardhwaj on liver MDA levels:** Liver MDA levels of normal control and Sidh Makardhwaj in the doses of 10, 50, 100 mg/kg treated groups were 64.66±1.48, 65.83±2.98, 66.72±2.63 and 92.70±6.03 nmol/g tissue, respectively. A significant increase in liver MDA levels at higher dose of Sidh Makardhwaj (100 mg/kg) treated group (P < 0.05) was observed while there was no significant change in liver MDA levels of Sidh Makardhwaj treated groups at lower doses (10 and 50 mg/kg) as compared to the normal control group (Fig 12.3.5).
Effect of chronic administration of Sidh Makardhwaj on GSH levels: GSH levels in liver tissue of normal control and Sidh Makardhwaj in the doses of 10, 50, 100 mg/kg treated groups were $3.31\pm0.21$, $3.28\pm0.13$, $3.15\pm0.07$ and $2.28\pm0.20$ mg/g tissue, respectively. A statistically significant decrease in liver GSH levels at higher dose of Sidh Makardhwaj (100 mg/kg) treated group ($P < 0.05$) was observed while there was no significant difference in liver GSH levels at lower doses Sidh Makardhwaj treated group at dose of 10 and 50 mg/kg as compared to normal control group (Fig 12.3.6).

Figure 12.3.5: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rat’s liver MDA level

Figure 12.3.6: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rat’s liver GSH level
*Effect of chronic administration of Sidh Makardhwaj on mercury levels in liver tissue:*  
Mercury level in liver tissues of normal control and Sidh Makardhwaj at the doses of 10, 50, 100 mg/kg treated groups were 46.49±5.64, 143.88±10.23, 198.88±8.61 and 302.73±8.56 µg/g wet tissue, respectively. There was a significant increase in liver mercury levels in higher doses Sidh Makardhwaj (50 and 100 mg/kg) treated groups (p< 0.001) as compared to normal control group. However, no statistically significant increase in liver mercury level was observed with the lower dose of Sidh Makardhwaj (10 mg/kg) as compared to the normal control group (Fig 12.3.4).

![Figure 12.3.4: Effect of Sidh Makardhwaj (10, 50 & 100 mg/kg) on rat’s liver mercury level](image)

*Effect of Sidh Makardhwaj on liver histology:* The liver of normal control and Sidh Makardhwaj (10 and 50 mg/kg) showed no abnormal histopathological changes but the liver of rats treated with higher dose of Sidh Makardhwaj (100 mg/kg) showed congestion of blood vessels, inflamed periportal zone, moderate inflammation of portal area macroscopically slight congestion of blood vessels. This histopathology showed nephrotoxicity only at the higher doses of Sidh Makardhwaj (100 mg/kg) but not at therapeutic dose (Fig 12.3.5).
Effect of Sidh Makardhwaj on rat’s organ to body weight ratio (%): Organ weights are widely accepted in the evaluation of toxic substance associated toxicities. The Society of Toxicologic Pathology (STP) recommends that organ weights should be included routinely in multi dose general toxicity studies with durations from 7 days to 1 year as Good Laboratory Practices (GLP). However, in the present study, there were no significant differences in the kidney, liver, brain, heart, lungs and spleen to body weight ratio (%) in Sidh Makardhwaj (10, 50, 100 mg/kg) treated groups as compared to the normal control group (Fig 12.3.6).
Effect of Arogyavardhini vati on rat’s brain, kidney and liver
12.4: Effect of Arogyavardhini vati on rat’s brain

*Effect of Arogyavardhini vati on passive avoidance task (step-through paradigm):* The mean initial latencies of normal control, Arogyavardhini vati of doses 50, 250 and 500 mg/kg were 13.9 ± 4.3, 12.8 ± 2.8, 13.9 ± 4.03, and 15.1 ± 5.7 s respectively and mean retention latencies on day 2\textsuperscript{nd} were 31.5 ± 2.2, 35.4 ± 2.4, 28.7 ± 3.5 and 44.7 ± 3.4, on day 7\textsuperscript{th} were 102.1 ± 2.4, 141.9 ± 2.6 and 172.9 ± 1.4 s, on day 14\textsuperscript{th} were 144.3 ± 0.6, 179.3 ± 1.1, 217.3 ± 1.09 and 200.2 ± 1.1 s and on day 28\textsuperscript{th} were 206.9 ± 1.07, 227.3 ± 0.9, 244.7 ± 0.4 and 258.6 ± 0.8 s. The results indicate that there were no significant differences among the mean retention latencies of all treated groups on day 2, 7, 14 and 28 as compared to normal group (Fig 12.4.1).

![Graph showing effect of Arogyavardhini vati on step through latency in rat.](image)

**Figure 12.4.1:** Effect of Arogyavardhini vati (50, 250 and 500 mg/kg) on step through latency in rat.

*Effect of Arogyavardhini vati on elevated plus maze:* The mean initial transfer latencies of normal control, Arogyavardhini vati of doses 50, 250 and 500 mg/kg were 41.1 ± 4.3, 44.0 ± 2.8, 41.7 ± 4.0 and 43.5 ± 5.7 s respectively and mean retention transfer latencies on day 2\textsuperscript{nd} were 10.3 ± 2.2, 13.0 ± 2.4, 12.0 ± 3.5 and 10.6 ± 2.9 s, on day 7\textsuperscript{th} were 10.5 ± 2.5, 7.7 ± 2.6, 7.6 ± 1.4 and 7.8 ± 1.3 s, on day 14\textsuperscript{th} were 8.1 ± 0.6, 5.5 ± 1.1, 5.7 ± 1.0 and 6.1 ± 1.1 s.
and on day 28th were 6.3 ± 1.0, 5.8 ± 0.9, 4.5 ± 0.4 and 5.0 ± 0.8 s. No significant difference was found among the mean retention latencies of all treated groups on day 2, 7, 14 and 28 as compared to normal group (Fig 12.4.2).

Effect of Arogyavardhini vati on Morris water maze: The mean distance traveled to reach platform by normal control, Arogyavardhini vati treated group of doses 50, 250 and 500 mg/kg during the acquisition trial were 17.1 ± 4.5, 18.7 ± 3.8, 13.5 ± 2.8 and 13.7 ± 2.6 m, whereas during the probe trial were 6.0 ± 1.8, 8.5 ± 2.1, 8.8 ± 1.5 and 7.9 ± 0.9 m, on day 7th were 5.8 ± 1.6, 7.0 ± 1.4, 5.4 ± 1.1 and 5.6 ± 1.3 m, on day 14th were 4.7 ± 1.1, 6.0 ± 1.0, 5.0 ± 1.0 and 4.2 ± 1.0 m and on day 28th were 3.7 ± 0.6, 4.4 ± 0.6, 3.3 ± 0.4 and 3.6 ± 0.8 m. No significant difference was observed in the mean distance traveled to reach platform among the Arogyavardhini vati treated as compared to normal group on day 0, 7, 14 and 28 (Fig 12.4.3).
Effect of Arogyavardhini vati on rota rod: The mean retention time of normal group, Arogyavardhini vati of doses 50, 250 and 500 mg/kg were 171.3 ± 21.1, 144.6 ± 16.8, 147.3 ± 17.5 and 137.8 ± 9.5 s respectively and mean retention transfer latencies on day 7th were 186.8 ± 15.9, 155.5 ± 13.5, 165.6 ± 13.8 and 158.8 ± 10.5 s, on day 14th were 187.6 ± 12.3, 152.8 ± 12.8, 176.8 ± 11.1 and 164.5 ± 8.4 s, on day 28th were 194.1 ± 13.6, 165.1 ± 12.3, 181.0 ± 9.3 and 176.6 ± 10.3 s. There were significant increased (p< 0.05) retention time on day 7th, 14th and 28th of normal control group and Arogyavardhini vati treated groups as compared to day 1st retention time. Arogyavardhini vati (50, 250 and 500 mg/kg, p.o) treatment for 28 days did not produced any significant decrease in the mean retention time ensuing restoration of motor coordination (Fig 12.4.4).
**Effect of Arogyavardhini vati rat brain MDA level:** The rat’s brain MDA levels of normal control and 3 doses of Arogyavardhini vati (50, 250 and 500 mg/kg) were 84.5 ± 4.2, 79.2 ± 3.8, 78.4 ± 3.0 and 72.0 ± 4.6 nmol/g-wet tissue respectively. The results of the present study shows that there were no significant difference in MDA level of Arogyavardhini vati (50, 250 and 500 mg/kg) treated groups as compared to normal group, shows no oxidative stress in Arogyavardhini vati treated group (Fig 12.4.5).

![Graph showing MDA levels](image)

**Figure 12.4.5:** Effect of Arogyavardhini vati (50, 250 & 500 mg/kg) on rat’s brain MDA level

**Effect of Arogyavardhini vati on rat brain GSH level:** The rat’s brain GSH levels of normal control, and 3 doses of Arogyavardhini vati (50, 250 and 500 mg/kg) were 2.23 ± 0.06, 2.31 ±0.04, 2.34 ± 0.04, 2.35 ± 0.03 mg/g-wet tissue respectively. The results of the present study shows that there were no significant difference in GSH level of Arogyavardhini vati (50, 250 and 500 mg/kg) treated groups as compared to normal group shows no oxidative stress in Arogyavardhini vati treated group (Fig 12.4.6).
Effect of Arogyavardhini vati on AChE activity of rat frontal cortex and hippocampus: The rat’s frontal cortex and hippocampus AChE activity of normal control, and 3 doses of Arogyavardhini vati (50, 250 and 500 mg/kg) were 24.98 ± 1.82, 24.47 ± 1.31, 24.49 ± 1.15, 23.22 ± 1.11 µM / g protein / min. and 29.17 ± 1.42, 28.89 ± 2.85, 28.57 ± 1.57, 27.85 ± 2.01 µM / g protein / min. respectively. The results of present study show that there were no significant difference in AChE activity of Arogyavardhini vati (50, 250 and 500 mg/kg) treated groups as compared to normal group in frontal cortex as well as in hippocampus (Fig 12.4.7 & 12.4.8).

Figure 12.4.6: Effect of Arogyavardhini vati on (50, 250 & 500 mg/kg) rat’s brain GSH level

Figure 12.4.7: Effect of Arogyavardhini vati (50, 250 & 500 mg/kg) on rat’s brain AChE activity in frontal cortex
Effect of Arogyavardhini vati on brain mercury level: The rat’s brain mercury levels of normal control and 3 doses of Arogyavardhini vati (50, 250 and 500 mg/kg) were 1.84±0.17, 7.11±1.12, 9.36±1.36, 13.34±1.90 µg/g-wet tissue respectively. The results of the present study shows significant increase in rat’s brain mercury levels of Arogyavardhini vati (50, 250 and 500 mg/kg) treated groups as compared to normal group (Fig 12.4.9).
Effect of Arogyavardhini vati on brain histology: The brain of normal control and Arogyavardhini vati treated groups (50, 250 and 500 mg/kg) showed normal cytoarchitecture (Fig 12.4.10).

![Effect of Arogyavardhini vati on rat's brain histology](image)

Figure 12.4.10: Effect of Arogyavardhini vati (50, 25 & 500 mg/kg) on rat’s brain histology

12.5: Effect of Arogyavardhini vati on kidney in rats

Effect of chronic administration of Arogyavardhini vati on serum urea nitrogen: Serum urea nitrogen level of normal control and Arogyavardhini vati in the doses of 50, 250, 500 mg/kg treated groups were 16.32±0.78, 17.70±1.76, 16.55±1.58, 14.80±1.28 mg/dl, respectively. As compared the normal control group, there was no significant difference in the serum urea nitrogen levels of Arogyavardhini vati treated group at doses of 50, 250 and 500 mg/kg (Fig 12.5.1).
Effect of chronic administration of Arogyavardhini vati on serum creatinine: Serum creatinine levels of normal control and Arogyavardhini vati in the doses of 50, 250, 500 mg/kg treated groups were 0.43 ± 0.03, 0.45 ± 0.04, 0.42 ± 0.04, 0.40 ± 0.03 mg/dl, respectively. As compared the normal control group, there was no significant difference in the serum creatinine levels of Arogyavardhini vati treated group at doses of 50, 250 and 500 mg/kg (Fig 12.5.2).

Figure 12.5.1: Effect of Arogyavardhini vati (50, 250 & 500 mg/kg) on rat’s serum urea nitrogen level

Figure 12.5.2: Effect of Arogyavardhini vati (50, 250 & 500 mg/kg) on rat’s serum creatinine level
Effect of chronic administration of Arogyavardhini vati on kidney MDA levels: Kidney MDA levels of normal control and Arogyavardhini vati in the doses of 50, 250, 500 mg/kg treated groups were 180.29±3.48, 183.87±3.22, 186.74±5.26, 191.04±3.75 nmol/g tissue, respectively. As compared the normal control group, there was no significant difference in kidney MDA levels of Arogyavardhini vati treated groups at doses of 50, 250 and 500 mg/kg (Fig 12.5.3).

![Graph showing effect of Arogyavardhini vati on rat's kidney MDA level](image)

Figure 12.5.3: Effect of Arogyavardhini vati (50, 250 & 500 mg/kg) on rat’s kidney MDA level

Effect of chronic administration of Arogyavardhini vati on GSH levels: GSH levels in kidney tissue of normal control and Arogyavardhini vati in the doses of 50, 250, 500 mg/kg treated groups were 4.02±0.09, 3.99±0.04, 3.85±0.03, 3.64±0.02 mg/g tissue, respectively. As compared the normal control group, there was no significant difference in kidney GSH levels of Arogyavardhini vati treated groups at doses of 50, 250 and 500 mg/kg (Fig 12.5.4).
Effect of chronic administration of Arogyavardhini vati on mercury levels in kidney tissue:

Mercury level in kidney tissues of normal control and Arogyavardhini vati in the doses of 50, 250, 500 mg/kg treated groups were 12.49±1.34, 23.68±3.78, 30.34±5.4, 34.31±2.64 µg/g wet tissue, respectively. There was a significant increase in kidney mercury levels at all doses of Arogyavardhini vati (50, 250 and 500 mg/kg) treated groups (p< 0.001) as compared to normal control group (Fig 12.5.5).
**Effect of Aroggyavardhini vati on kidney histology:** The kidney of normal control and Aroggyavardhini vati treated groups (50, 250 and 500 mg/kg) showed normal cytoarchitecture (Fig 12.5.6).

![Kidney histology images](image)

Figure 12.5.6: Effect of Aroggyavardhini vati (50, 250 & 500 mg/kg) on rat’s kidney histology

**12.6: Effect of Aroggyavardhini vati on liver in rats**

**Effect of chronic administration of Aroggyavardhini vati on serum ALT:** Serum ALT level of normal control and Aroggyavardhini vati in the doses of 50, 250, 500 mg/kg treated groups were 129.48±1.39, 127.75±1.82, 126.25±2.29, 127.67±2.05 IU/l, respectively. As compared the normal control group, there was no significant difference in the serum ALT levels of Aroggyavardhini vati treated group at doses of 50, 250 and 500 mg/kg (Fig 12.6.1).

![Serum ALT levels](image)

Figure 12.6.1: Effect of Aroggyavardhini vati (50, 250 & 500 mg/kg) on rat’s serum ALT level
**Effect of chronic administration of Arogyavardhini vati on serum AST:** Serum AST level of normal control and Arogyavardhini vati in the doses of 50, 250, 500 mg/kg treated groups were 106.50±1.74, 105.08±1.88, 104.10±1.83, 103.68±1.25 IU/l, respectively. As compared the normal control group, there was no significant difference in the serum AST levels of Arogyavardhini vati treated group at doses of 50, 250 and 500 mg/kg (Fig 12.6.2).

![Figure 12.6.2: Effect of Arogyavardhini vati (50, 250 & 500 mg/kg) on rat’s serum AST level](image)

**Effect of chronic administration of Arogyavardhini vati on serum ALP:** Serum ALP level of normal control and Arogyavardhini vati in the doses of 50, 250, 500 mg/kg treated groups were 41.92±0.53, 42.52±0.86, 40.95±0.79, 39.75±0.77 IU/l, respectively. As compared the normal control group, there was no significant difference in the serum ALP levels of Arogyavardhini vati treated group at doses of 50, 250 and 500 mg/kg (Fig 12.6.3).

![Figure 12.6.3: Effect of Arogyavardhini vati (50, 250 & 500 mg/kg) on rat’s serum ALP level](image)
Effect of chronic administration of Arogyavardhini vati on serum bilirubin: Serum bilirubin level of normal control and Arogyavardhini vati in the doses of 50, 250, 500 mg/kg treated groups were 0.13±0.01, 0.16±0.01, 0.14±0.02, 0.11±0.01 mg/dl, respectively. As compared the normal control group, there was no significant difference in the serum bilirubin levels of Arogyavardhini vati treated group at doses of 50, 250 and 500 mg/kg (Fig 12.6.4).

![Graph showing serum bilirubin levels](image)

Figure 12.6.4: Effect of Arogyavardhini vati (50, 250 & 500 mg/kg) on rat’s serum bilirubin level

Effect of chronic administration of Arogyavardhini vati on liver MDA levels: Liver MDA levels of normal control and Arogyavardhini vati in the doses of 50, 250, 500 mg/kg treated groups were 64.66±1.48, 67.50±1.90, 67.73±2.28 and 69.11±1.80 nmol/g tissue, respectively. As compared the normal control group, there was no significant difference in the MDA levels of Arogyavardhini vati treated group at doses of 50, 250 and 500 mg/kg (Fig 12.6.5).

![Graph showing liver MDA levels](image)

Figure 12.6.5: Effect of Arogyavardhini vati (50, 250 & 500 mg/kg) on rat’s liver MDA level
**Effect of chronic administration of Arogyavardhini vati on liver GSH levels:** GSH levels in liver tissue of normal control and Arogyavardhini vati in the doses of 50, 250, 500 mg/kg treated groups were 3.31±0.21, 3.17±0.25, 3.15±0.07 and 3.06±0.29 mg/g tissue, respectively. As compared the normal control group, there was no significant difference in the serum bilirubin levels of Arogyavardhini vati treated group at doses of 50, 250 and 500 mg/kg (Fig 12.6.6).

![Liver GSH levels](image)

**Figure 12.6.6:** Effect of Arogyavardhini vati (50, 250 & 500 mg/kg) on rat’s liver GSH level

**Effect of chronic administration of Arogyavardhini vati on mercury levels in liver tissue:**
Mercury level in liver tissues of normal control and Arogyavardhini vati in the doses of 50, 250, 500 mg/kg treated groups were 13.40±1.03, 21.01±2.98, 26.50±1.89 and 27.83±3.21µg/g wet tissue, respectively. There was a significant increase in liver mercury levels at all doses of Arogyavardhini vati (50, 250 and 500 mg/kg) treated groups (p< 0.001) as compared to normal control group (Fig 12.6.7).
Figure 12.6.7: Effect of Arogyavardhini vati (50, 250 & 500 mg/kg) on rat’s liver mercury level

Effect of Arogyavardhini vati on liver histology: The kidney of normal control and Arogyavardhini vati treated groups (50, 250 and 500 mg/kg) showed normal cytoarchitecture (Fig 12.6.8).

Figure 12.6.8: Effect of Arogyavardhini vati (50, 250 & 500 mg/kg) on rat’s liver histology
Effect of Arogyavardhini vati on rat’s organ to body weight ratio (%): Organ weights are widely accepted in the evaluation of toxic substance associated toxicities. The Society of Toxicologic Pathology (STP) recommends that organ weights should be included routinely in multi dose general toxicity studies with durations from 7 days to 1 year as Good Laboratory Practices (GLP). However, in the present study, there were no significant differences in the kidney, liver, brain, heart, lungs and spleen to body weight ratio (%) in Arogyavardhini vati (50, 250, 500 mg/kg) treated groups as compared to the normal control group (Fig 12.6.9).

![Figure 12.6.9: Effect of Arogyavardhini vati (50, 250 & 500 mg/kg) on rat’s organ to body weight ratio (%).]
12.7: Effect of Ayurvedic treatment (Ashwagandha powder and Sidh Makardhwaj) in rheumatoid arthritis patients

**Patient characteristics:** A total of 90.69% (78/86) patients completed 7 weeks of treatment and 9.3% (8/86) prematurely discontinued due to lack of efficacy and refusal of continued treatment. Female and male rheumatoid arthritis (RA) patients were 57.7% (45/78) and 42.3% (33/78) respectively and mean age were 45.7±8.6 (range 19-59) and 49.8±7.9 (range, 25-59) years respectively. At baseline, majority of patients tested positive for rheumatoid factor (RF) and (ESR). Male and female patient’s baseline levels of ESR and RA factor were 28.8±3.3 & 31.2±3.1 and 43.8±16.16.3 & 50.9±16.1 respectively and post treatment levels were 21.6±1.9 & 22.1±1.4 and 35.4±14.3 & 41.7 ±14.4. There were significant decrease in post treatment levels of ESR and RA factor as compared to baseline levels in male and female.

Tender joint counts, swollen joint counts, physician global assessment score, patient global assessment score, pain assessment score, patient self assessed disability index score, visual analogue scale (VAS) score at baseline in male patients were 6.55±1.27, 3.36±1.73, 46.36±7.83, 52.12±11.11, 3.3±1.09, 6.21±0.66, 58.79±8.2 respectively and in female patients were 6.6±1.21, 3.87±1.80, 47.56±8.02, 53.56±11.52, 3.32±1.25, 6.17±0.72, 58.89±8.85 respectively and at post treatment in male patients were 4.82±0.81, 2.45±1.03, 33.64±6.99, 35.15±7.95, 2.47±0.91, 4.41±0.42, 41.21±7.39 respectively and female patients were 4.75±0.55, 2.73±1.01, 6.0±6.54, 34.44±7.85, 2.57±0.91, 4.44±0.48, 40.44±7.37 respectively. There were significant change in post treatment scores of tender joint counts, swollen joint counts, physician global assessment score, patient global assessment score, pain assessment score, patient self assessed disability index score, visual analogue scale (VAS) score as compared to baseline scores in male and female patients.
In our study, DAS28 score in male and female patients at baseline were 5.01±0.36 & 5.12±0.33 respectively and at post treatment were 4.29±0.21 & 4.28±0.19. ACR 20 criteria were achieved by 48.5% (16/33) male and 62.2% (28/45) female patients (Table 12.7.2)

**Table: 12.7.2: Clinical features in rheumatoid arthritis patients**

<table>
<thead>
<tr>
<th>Disease parameters</th>
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<th>Base line</th>
<th>Post treatment</th>
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<td>Swollen joint counts</td>
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<td>5.12±0.33</td>
<td>4.28±0.19***</td>
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</tbody>
</table>

Data are expressed as mean ± SEM, *** p<0.01 as compared to baseline values.
A DAS28 score of higher than 5.1 is indicative of high disease activity, whereas a DAS28 below 3.2 indicates low disease activity. 57.7% (45/78) patients were in moderate disease activity with DAS28 score between 3.2 and 5.1. High disease activity was observed in 42.3% (33/78) patients with DAS28 score >5.1 (Table 12.7.3).

Table 12.7.3: Efficacy of Ashwagandha and Sidh Makardhwaj treatment in rheumatoid arthritis patients (DAS28 improvement score)

<table>
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<th>Present DAS 28</th>
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<td>Moderate response</td>
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<td></td>
<td>3.2-5.1 (Moderate disease activity)</td>
<td>(n= 0)</td>
<td>Moderate response</td>
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<td></td>
<td>&gt; 5.1 (High disease activity)</td>
<td>(n= 06)</td>
<td>Moderate response</td>
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</table>

In moderate disease activity, DAS28 improvement over the time in the range of 0.6-1.2 was observed in 55.6% (25/45) patients and <0.6 improvement over the time in DAS28 was observed in 44.4% (20/45) patients. In high disease activity, >1.2 improvement over the time in DAS28 was observed in 18.2% (6/33) patients and DAS28 improvement over the time in the range of 0.6-1.2 was observed in 81.8% (27/33) patients.

In Summary, Sidh Makardhwaj treatment for 28 days in rheumatoid arthritis patients showed moderated response in 39.74% (31/78) patients.
12.8: Evaluation of safety of Ayurvedic treatment (Ashwagandha powder and Sidh Makardhwaj) in rheumatoid arthritis patients

The serum ALT, AST, ALP, bilirubin, urea and creatinine levels on visit 1st, 2nd, 3rd and 4th visit were analyzed in both male and females (Table 12.8.1). However, early marker of renal damage i.e. Serum beta-2 microglobulin (β2MG) and Neutrophil Gelatinase Associated Lipocalin (NGAL) levels, were analyzed at baseline and post treatment (Table 12.8.2).

Table 12.8.1: Effect on levels of serum ALT, AST, ALP, Bilirubin, Creatinine, & urea of rheumatoid arthritis patients on treatment with Ashwagandha and Sidh makardhwaj.

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<td></td>
<td>F</td>
<td>0.73±0.16</td>
<td>0.75±0.16</td>
<td>0.77±0.16</td>
<td>0.80±0.16</td>
</tr>
<tr>
<td>Urea</td>
<td>M</td>
<td>27.46±5.04</td>
<td>28.23±4.92</td>
<td>28.91±4.74</td>
<td>30.01±5.21</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>27.58±5.77</td>
<td>28.37±5.48</td>
<td>28.92±5.41</td>
<td>29.69±5.18</td>
</tr>
<tr>
<td>Creatinine</td>
<td>M</td>
<td>0.79±0.10</td>
<td>0.82±0.10</td>
<td>0.84±0.11</td>
<td>0.88±0.10</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.87±0.09</td>
<td>0.83±0.09</td>
<td>0.85±0.03</td>
<td>0.88±0.10</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
Table 12.8.2: Effect on levels of serum B2MG and NGAL of rheumatoid arthritis patients on treatment with Ashwagandha and Sidh makardhwaj.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Sex</th>
<th>Baseline</th>
<th>Post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>1.07±0.41</td>
<td>1.12±0.38</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1.05±0.41</td>
<td>1.27±0.38</td>
</tr>
<tr>
<td>B2MG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>35.16±8.5</td>
<td>35.79±8.40</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>35.52±9.53</td>
<td>36.09±8.90</td>
</tr>
<tr>
<td>NGAL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was no significant change in serum levels of male and female ALT, AST, ALP, bilirubin, urea, creatinine, β2MG and NGAL at post treatment as compared to baseline.

12.9: Effect of Ayurvedic treatment (Arjuna powder and Arogyavardhini vati) in dyslipidemia patients

The serum levels of total cholesterol, LDL, HDL, triglycerides and CRP were determined at the start and at the end of treatment. There was a significant reduction in total cholesterol, LDL and triglycerides, whereas there was a significant elevation in the HDL level. The fall in CRP levels was quite significant at around 27.6% (Table 12.9.1). Statistical analysis of the results confirmed the significance of the above observation with the reduction in total cholesterol, LDL and triglycerides (p<0.01) and HDL elevation (p< 0.05). The percentage decrease in the levels of serum total cholesterol, LDL, TG were 9.8%, 8.8%, 9.9% respectively and increased HDL was 8.1%. There was a significant fall in blood glucose showing better glucose metabolism (Table 12.9.1).
Table 12.9.1: Lipid profile, CRP and Blood glucose value of dyslipidemia patients.

Data are expressed as mean±SD, *** p<0.001 as compared to baseline levels

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>Post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>236.4±15.1</td>
<td>213.2±14.7***</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dL)</td>
<td>162.9±12.8</td>
<td>148.3±10.4***</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>219.9±26.9</td>
<td>198.1±21.5***</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dL)</td>
<td>39.9±4.1</td>
<td>43.14±3.2***</td>
</tr>
<tr>
<td>C-Reactive Protein (mg/L)</td>
<td>2.9±0.9</td>
<td>2.1±0.8***</td>
</tr>
<tr>
<td>Blood Glucose (mg/dL)</td>
<td>106.3±15.3</td>
<td>91.8±11.6***</td>
</tr>
</tbody>
</table>

12.10: Safety evaluation of Arjuna and Arogyavardhini vati in dyslipidemia patients

Safety of these Ayurvedic formulations is an added feature. There were no significant change in serum ALT, AST, ALP and bilirubin, urea and creatinine at the end of study as compared to baseline levels (Table 12.10.1). Early sensitive marker of liver (β2 microglobulin) and kidney (NGAL: Neutrophil Gelatinase Associated Lipocalin) showed no significant change.
Table 12.10.1: Safety evaluation of liver and kidney of dyslipidemia patients on treatment with Arjuna powder and Arogyavardhini vati

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Visit 1 (Day 0)</th>
<th>Visit 2 (Day 21)</th>
<th>Visit 3 (Day 35)</th>
<th>Visit 4 (Day 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>28.5±4.6</td>
<td>25.8±3.5</td>
<td>28.1±3.3</td>
<td>30.2±3.4</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>28.8±4.1</td>
<td>27.6±3.9</td>
<td>28.9±4.0</td>
<td>30.3±4.3</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>142.6±17.9</td>
<td>141.1±17.7</td>
<td>143.4±16.5</td>
<td>144.6±17.8</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.80±0.18</td>
<td>0.84±0.17</td>
<td>0.86±0.18</td>
<td>0.88±0.16</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>28.5±5.3</td>
<td>28.9±5.2</td>
<td>29.3±5.3</td>
<td>29.8±5.2</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.83±0.10</td>
<td>0.83±0.09</td>
<td>0.85±0.11</td>
<td>0.89±0.14</td>
</tr>
<tr>
<td>B2MG (µg/ml)</td>
<td>1.08±0.4</td>
<td></td>
<td>1.11±0.3</td>
<td></td>
</tr>
<tr>
<td>NGAL (µg/ml)</td>
<td>36.4±9.2</td>
<td></td>
<td>36.2±7.1</td>
<td></td>
</tr>
</tbody>
</table>
12.11: Evaluation of cytotoxicity of Mercury, Gold & Sidh makardhwaj & Arogyavardhini vati on HepG2 cells using MTT assay

Effect of mercury on HepG2 cell line

The effects of mercury on the viability of HepG2 cells are shown in Figure 12.11.1. There was statistically significant decrease in cell viability with increasing dose and exposure time. Data presented in Figure 12.11.1 indicate a significant dose and time dependent toxicity of mercury at 1.65, 3.12, 6.25, 12.5, 25, 50 and 100 µg/ml for 3, 6, 12 and 24 hrs. Mercury exposure for 24 hours at 100 µg/ml showed only 7.6 ± 1.1 % of cell viability, indicating that mercury is highly toxic to the cells. High doses of mercury (12.5, 25, 50 and 100 µg/ml) showed significant decreased in cell viability with increasing time exposure (p<0.001). However, there was no significant change in cell viability observed at low doses of mercury (1.65, 3.12 and 6.25 µg/ml) with increasing time exposure. Moreover, cell proliferation was observed at low dose of mercury (1.65 µg/ml) at 3, 6, 12 and 24 h (Figure12.11.1).

![Bar chart showing dose and time dependent effect of mercury on HepG2 cell line](image)

Figure 12.11.1: Dose and time dependent effect of mercury on HepG2 cell line. Data are expressed as mean ± S.E.M. *** p < 0.001, as compared to low dose of mercury (1.65 µg/ml) at 3, 6, 12 and 24 hrs.
Effect of gold on HepG2 cell line

Dose and time dependent effect of gold on HepG2 cells are shown in Figure 12.11.2. There was no statistically significant change in cell viability at low dose of gold (1.65, 3.12, 6.25 µg/ml). A dose and time dependent decrease in cell viability was observed at higher doses of gold (12.5, 25, 50 and 100 µg/ml) with increasing exposure duration (3, 6, 12 and 24 hrs). Gold exposure for 24 hours at 100 µg/ml showed 31.8 ± 2.1 % of cell viability, indicating that gold is toxic to HepG2 cells. There was no significant change in cell viability observed at low doses of mercury (1.65, 3.12 and 6.25 µg/ml) with increasing time exposure. Moreover, cell proliferation was observed at 1.65, 3.12, 6.25 µg/ml of gold exposure (Figure 12.11.2).

![Figure 12.11.2: Dose and time dependent effect of gold on HepG2 cell line. Data are expressed as mean ± S.E.M. *** p < 0.001, as compared to low dose of gold (1.65 µg/ml) at 3, 6, 12 and 24 hrs.](image-url)
Effect of mercury and gold (Hg + Au) solution on HepG2 cell line

Mercury and gold coexposure with high doses (12.5, 25, 50 and 100 µg/ml) at 3, 6, 12 and 24 h showed statistically significant decreased the cell viability of HepG2 cells. Co exposure of these two metals for 24 hours at 100 µg/ml showed only 10.1 ± 1.7 % of cell viability, indicating that coexposure is highly toxic to HepG2 cells. However, there was no statistically significant change in cell viability observed at low doses of mercury and gold solution (1.65, 3.12 and 6.25 µg/ml) with increasing time exposure. Moreover, cell proliferation was observed at 1.65, 3.12 and 6.25 µg/ml of mercury and gold solution (Figure 12.11.3).

**Figure 12.11.3: Dose and time dependent effect of mercury and gold (Hg + Au) solution on HepG2 cell line.** Data are expressed as mean ± S.E.M. ***p < 0.001, as compared to low dose of mercury and gold solution (1.65 µg/ml) at 3, 6, 12 and 24 hrs.

Effect of Sidh Makardhwaj on HepG2 cell line

Exposure of HepG2 cell with Sidh Makardhwaj at doses of 20, 40, 80 and 200 µg/ml for 3, 6, 12 and 24 h did not showed statistically significant change in cell viability. Sidh Makardhwaj exposure for 24 hours at maximum dose (200 µg/ml) showed 96.2 ± 5.0 % of cell viability, indicating that Sidh Makardhwaj is nontoxic to HepG2 cells. However, cell proliferation was observed at 20 µg/ml of Sidh Makardhwaj exposure at 3 hrs (Figure 12.11.4).
Figure 12.11.4: Dose and time dependent effect of Sidh Makardhwaj on HepG2 cell line. Data are expressed as mean ± S.E.M.

Effect of Arogyavardhini on vati HepG2 cell line

The exposure of HepG2 cell with Arogyavardhini vati at 100, 200, 400 and 800 µg/ml, for 3, 6, 12 and 24 h did not showed statistically significant change the percentages of cell viability. Arogyavardhini vati exposure for 24 hours at 100 µg/ml showed 110.2 ± 8.2 % of cell viability, indicating that Arogyavardhini vati is not cytotoxic to HepG2 cells. However, cell proliferation was observed at 800 µg/ml of Arogyavardhini vati exposure at 6, 12 and 24 hrs (Figure 12.11.5).

Figure 12.11.5: Dose and time dependent effect of Arogyavardhini vati on HepG2 cell line. Data are expressed as mean ± S.E.M.
Effect of Ras Sindur on vati HepG2 cell line

Exposure of HepG2 cell with Ras Sindur at doses of 25, 50, 100 and 200 µg/ml for 3, 6, 12 and 24 h did not showed statistically significant change in cell viability. Ras Sindur exposure for 24 hours at maximum dose (200 µg/ml) showed 101.5 ± 4.1 % of cell viability, indicating that Ras Sindur is nontoxic to HepG2 cells (Figure 12.11.6).

![Graph showing cell viability of HepG2 cells exposed to different doses of Ras Sindur for various periods of time.](image)

**Figure 12.11.6: Dose and time dependent effect of Ras Sindur on HepG2 cell line.** Data are expressed as mean ± S.E.M.

Effect of Kajjali on HepG2 cell line

Exposure of HepG2 cell with Kajjali at doses of 50, 100, 200 and 400 µg/ml for 3, 6, 12 and 24 h did not showed statistically significant change in cell viability. Kajjali exposure for 24 hours at maximum dose (200 µg/ml) showed 97.8 ± 6.1 % of cell viability, indicating that Kajjali is nontoxic to HepG2 cells (Figure 12.11.7).
Figure 12.11.7: Dose and time dependent effect of Kajjali on HepG2 cell line. Data are expressed as mean ± S.E.M.

12.12: Evaluation of cytotoxicity of Mercury, Gold & Sidh makardhwaj & Arogyavardhini vati on HEK cell line using MTT assay

Effect of mercury on HEK cell line

The effects of mercury on the viability of HEK cells are shown on Figure 12.12.1. There was statistically significant decrease in cell viability with increasing dose and exposure time. Data presented in figure 3.8 indicate a significant dose and time dependent toxicity of mercury at 1.65, 3.12, 6.25, 12.5, 25, 50 and 100 µg/ml for 3, 6, 12 and 24 hrs. Mercury exposure for 24 hours at 100 µg/ml showed only 9.1 ± 2.1 % of cell viability, indicating that mercury is highly toxic to the cells. High doses of mercury (3.12, 6.25, 12.5, 25, 50 and 100 µg/ml) showed significant decreased in cell viability with increasing time exposure (p<0.001). However, there was no significant change in cell viability observed at low doses of mercury (1.65, 3.12 and 6.25µg/ml) with increasing time exposure (Figure 12.12.1).
**Figure 12.12.1:** Dose and time dependent effect of mercury on HEK cell line. Data are expressed as mean ± S.E.M. ***$p < 0.001$, as compared to low dose of mercury (1.65 µg/ml) at 3, 6, 12 and 24 hrs.

**Effect of gold on HEK cell line**

Dose and time dependent effect of gold on HEK cells are shown in Figure 12.12.2. There was no statistically significant change in cell viability at low dose of gold (1.65, 3.12, 6.25, 12.5 µg/ml). A dose and time dependent decrease in cell viability was observed at higher doses of gold (25, 50 and 100 µg/ml) with increasing exposure duration (3, 6, 12 and 24 hrs). Gold exposure for 24 hours at 100 µg/ml showed 15.3 ± 5.1 % of cell viability, indicating that gold is toxic to HepG2 cells. There was no significant change in cell viability observed at low doses of mercury (1.65, 3.12 and 6.25 µg/ml) with increasing time exposure. Moreover, cell proliferation was observed at 1.65 and 3.12 µg/ml of gold exposure (Figure 12.12.2).
Figure 12.12.2: Dose and time dependent effect of gold on HEK cell line. Data are expressed as mean ± S.E.M. *** $p < 0.001$, as compared to low dose of gold (1.65 µg/ml) at 3, 6, 12 and 24 hrs.

Effect of mercury and gold (Hg + Au) solution on HEK cell line

Mercury and gold co-exposure with high doses (3.12, 6.25, 12.5, 25, 50 and 100 µg/ml) at 3, 6, 12 and 24 h showed statistically significant decreased the cell viability of HEK cells. Co-exposure of these two metals for 24 hours at 100 µg/ml showed only 6.8 ± 1.9 % of cell viability, indicating that co-exposure is highly toxic to HEK cells. However, there was no statistically significant change in cell viability observed at low doses of mercury and gold solution (1.65, µg/ml). Moreover, cell proliferation was observed at 1.65 µg/ml of mercury and gold solution (Figure 12.12.3).
Figure 12.12.3: Dose and time dependent effect of mercury and gold solution on HEK cell line. Data are expressed as mean ± S.E.M. *** $p < 0.001$, as compared to low dose of gold (1.65 µg/ml) at 3, 6, 12 and 24 hrs.

Effect of Sidh Makardhwaj on HEK cell line

Exposure of HEK cell with Sidh Makardhwaj at doses of 20, 40, 80 and 200 µg/ml for 3, 6, 12 and 24 h did not showed statistically significant change in cell viability. Sidh Makardhwaj exposure for 24 hours at maximum dose (200 µg/ml) showed 101.9 ± 5.9 % of cell viability, indicating that Sidh Makardhwaj is nontoxic to HEK cells. However, cell proliferation was observed at 20 µg/ml of Sidh Makardhwaj exposure at 12 and 24 hrs (Figure 12.12.4).

Figure 12.12.4: Dose and time dependent effect of Sidh Makardhwaj on HEK cell line

Values are expressed as mean ± S.E.M.
Effect of Arogyavardhini vati on HEK cell line

The exposure of HEK cell with Arogyavardhini vati at 100, 200, 400 and 800 µg/ml for 3, 6, 12 and 24 h did not showed statistically significant change the percentages of cell viability. Arogyavardhini vati exposure for 24 hours at 100 µg/ml showed 106.0 ± 8.1 % of cell viability, indicating that Arogyavardhini vati is not cytotoxic to HEK cells. However, cell proliferation was observed at 100, 200, 400 and 800 µg/ml of Arogyavardhini vati exposure at 3, 6, 12 and 24 hrs (Figure 12.12.5).

![Graph showing dose and time dependent effect of Arogyavardhini vati on HEK cell line](image)

**Figure 12.12.5: Dose and time dependent effect of Arogyavardhini vati on HEK cell line**

Values are expressed as mean ± S.E.M.

12.13: Effect of Ras Sindur on HEK cell line

Exposure of HEK cell with Ras Sindur at doses of 25, 50, 100 and 200 µg/ml for 3, 6, 12 and 24 h did not showed statistically significant change in cell viability. Ras Sindur exposure for 24 hours at maximum dose (200 µg/ml) showed 98.4 ± 3.3 % of cell viability, indicating that Ras Sindur is nontoxic to HEK cells (Figure 12.13.1).
Effect of Kajjali on HEK cell line

Exposure of HEK cell with Kajjali at doses of 50, 100, 200 and 400 µg/ml for 3, 6, 12 and 24 h did not showed statistically significant change in cell viability. Kajjali exposure for 24 hours at maximum dose (200 µg/ml) showed 94.6 ± 3.7 % of cell viability, indicating that Kajjali is nontoxic to HEK cells (Figure 12.13.2).

Figure 12.13.1: Dose and time dependent effect of Ras Sindur on HEK cell line. Data are expressed as mean ± S.E.M.

Figure 12.13.2: Dose and time dependent effect of Kajjali on HEK cell line. Data are expressed as mean ± S.E.M.
13. Detailed analysis of results indicating contributions made towards increasing the state of knowledge in the subject.

I. Experimental study

Followings are the summary results of experimental animal study

A) Sidh Makardhwaj

- Sidh Makardhawaj (10, 50 and 100 mg/kg) treated groups did not show statistical significant change in retention latencies of passive avoidance task, retention latencies of elevated plus maze, retention time on rota rod on day 1, 2, 7, 14 and 28 as compared to normal control. However, mercuric chloride treated group showed significant decrease in mean retention latencies on day 7, 14 and 28 as compared to normal control group ($p < 0.001$).

- There was no significant change in mean distance traveled to reach platform on day 1, 4, 7, 14 and 28 of Sidh Makardhawaj (10, 50 and 100 mg/kg) treated groups as compared to normal control. However, mercury chloride treated group showed significant increase in mean distance traveled to reach platform on day 4, 7, 14 and 28 compared to normal control group ($p < 0.001$).

- Rat’s brain AChE activity of Sidh Makardhwaj (10, 50 and 100 mg/kg) treated groups did not show significant change as compared to normal group in frontal cortex as well as in hippocampus. However, there was significant ($p < 0.001$) decrease in brain AChE activity in frontal cortex as well as in hippocampus of mercury chloride treated group as compared to normal control group.

- Rat’s brain MDA and GSH level did not show significant change at all doses of Sidh Makardhawaj as compared to normal control. Higher dose Sidh Makardhwaj (100 mg/kg) and mercuric chloride treated group showed significant increased MDA and decreased GSH levels in rat’s liver and kidney ($p<0.001$).
Sidh Makardhwaj (10, 50 and 100 mg/kg) treated group did not show significant change in the serum ALT, AST, ALP, bilirubin, urea and creatinine levels as compared the normal control group, while significant change was observed in mercuric chloride treated group (p<0.001).

Rat’s brain, liver and kidney mercury levels of Sidh Makardhwaj (10, 50 and 100 as well as in mercury chloride treated group was significantly increased (p < 0.001) as compared to normal group.

The brain, liver and kidney of normal control group and lower doses of Sidh Makardhwaj (10, 50 mg/kg) showed normal histology but mild histopathological change was observed with higher dose of Sidh Makardhwaj (100 mg/kg). However, necroses of neurons in brain, pyknosis, vascular congestion, degenerative and necrosis in liver, congestion of blood vessels and epithelium disruption in proximal convoluted tubules of kidney was observed in mercury chloride treated rats.

The findings of the present study suggest that Sidh Makardhwaj upto 5 times the equivalent dose administered to rats for 28 days did not show any toxicological effects on brain, liver and kidney.

B) Arogyavardhini vati

Arogyavardhini vati (50, 250 and 500 mg/kg) treated groups did not show statistical significant change in retention latencies of passive avoidance task, retention latencies of elevated plus maze, retention time on rota rod on day 1, 2, 7, 14 and 28 as compared to normal control.

Arogyavardhini vati (50, 250 and 500 mg/kg) treated groups did not show statistical significant change in mean distance traveled to reach platform on day 1, 4, 7, 14 and 28 as compared to normal control.
Rat’s brain AChE activity of Arogyavardhini vati (50, 250 and 500 mg/kg) treated groups did not show significant change as compared to normal group in frontal cortex as well as in hippocampus.

Rat’s brain, liver, kidney MDA and GSH level did not show significant change at all doses of Arogyavardhini vati (50, 250 and 500 mg/kg) as compared to normal control.

Arogyavardhini vati (50, 250 and 500 mg/kg) treated group did not show significant change in the serum ALT, AST, ALP, bilirubin, urea and creatinine levels as compared the normal control group.

Rat’s brain, liver and kidney mercury levels of Arogyavardhini vati (50, 250 and 500 mg/kg) treated group was significantly increased (p < 0.001) as compared to normal group.

The brain, liver and kidney of normal control group and all doses of Arogyavardhini vati (50, 250 and 500 mg/kg) showed normal cytoarchitecture.

The findings of the present study suggest that Arogyavardhini vati upto 10 times the equivalent dose administered to rats for 28 days did not show any toxicological effects on brain, liver and kidney.

II. Clinical study

As per WHO guidelines, clinical evaluation of herbal medicines attempt to recognize the long and diverse history of traditional medicine in the Region and the differences between the diagnostic systems of modern medicine and the various traditional medicines. Although special considerations may be required, the general principles of the clinical trials of herbal medicines are similar to those applied to synthetic drugs if clinical trial is regarded to be necessary. Clinical trials of herbal medicines may have two types of objectives. One is to validate the safety and efficacy that is claimed for a traditional herbal medicine. The other is
to develop new herbal medicines or examine a new indication for an existing herbal medicine or a change of dose formulation, or route of administration.

In the present study, clinical trial of Sidh Makardhwaj and Arogyavardhini vati was done with the objective is to validate the safety and efficacy that is claimed for a traditional herbal medicine. These two drugs are mercury based formulation used for centuries but their safety was ascertained. Doses were selected as per the Ayurvedic text (Ayurvedic Formulary of India) and efficacy was evaluated.

A pilot prospective cohort clinical study was designed to evaluate the safety and efficacy of Ashwagandha powder & Sidh Makardhwaj for rheumatoid arthritis and Arjuna powder & Arogyavardhini vati for dyslipidemia patients.

**Sidh Makardhwaj for rheumatoid arthritis**

- A total of 90.7% (78/86) rheumatoid arthritis patients adhered to study protocol. There were 57.7% (45/78) female and 42.3% (33/78) male subjects and mean age were 45.7±8.6 (range 19-59) and 49.8±7.9 (range, 25-59) years respectively. At baseline, majority of patients tested positive for rheumatoid factor (RF).
- There were statistically significant change in post treatment scores of tender joint counts, swollen joint counts, physician global assessment score, patient global assessment score, pain assessment score, patient self assessed disability index score and ESR level as compared to baseline scores in male and female patients.
- ACR 20 response was observed in 48.5% (16/33) male and 62.2% (28/45) female.
- There was no significant change in serum levels of male and female ALT, AST, ALP, bilirubin, urea, creatinine, β2MG and NGAL at post treatment as compared to baseline.
Urinary mercury level increased significantly after Ayurvedic treatment as compared to baseline level.

The findings of the present study suggest that Sidh Makardhwaj is safe and effective. The present Ayurvedic treatment (Ashwagandha powder and Sidh Makardhwaj) can thus potentially be used for the treatment of rheumatoid arthritis.

**Arogyavardhini vati for dyslipidemia patients**

A total of 86.1% (87/101) dyslipidemia patients adhered to study protocol. There were 65.5% (57/87) men 14.5% (30/87) female.

There was a significant reduction in total cholesterol, LDL and triglycerides, whereas there was a significant elevation in the HDL level. The percentage decrease in the levels of serum total cholesterol, LDL, TG were 9.8%, 8.8%, 9.9% respectively and increased HDL was 8.1%. There was a significant fall in blood glucose showing better glucose metabolism.

The fall in CRP levels was quite significant at around 27.6%.

There was no significant change in serum ALT, AST, ALP and bilirubin, urea and creatinine at the end of study as compared to baseline levels.

Early sensitive marker of liver (β2 microglobulin) and kidney (NGAL: Neutrophil Gelatinase Associated Lipocalin) showed no significant change.

The findings of the present study suggest that Arogyavardhini vati is safe and effective. In conclusion, results of the present prospective cohort study showed that Ayurvedic treatment (Arjuna powder and Arogyavardhini) is safe and effective for dyslipidemia patients.
III. Cell culture study

Mercury exposure at 12.5, 25, 50 and 100 µg/ml in HepG2 cells showed statistically significant decreased in cell viability with increasing time exposure (p<0.001). However, significant decreased in cell viability at the doses of 3.12, 6.25, 12.5, 25, 50 and 100 µg/ml was shown in HEK cell line (p<0.001).

- There was statistically significant decrease in cell viability of HepG2 cells at 12.5, 25, 50 and 100 µg/ml of gold. However, HEK cell line exposure at higher doses of gold (25, 50 and 100 µg/ml) showed statistically significant decrease in cell viability (p<0.001) and 100% cells were viable at lower doses.

- Mercury and gold co exposure significantly decreased the cell viability at doses of 12.5, 25, 50 and 100 µg/ml in HepG2 cells (p<0.001). However, decreased cell viability was observed at the doses of 3.12, 6.25, 12.5, 25, 50 and 100 µg/ml in HEK cells (p<0.001).

- Exposure of HepG2 and HEK cells with Sidh Makardhwaj at doses of 20, 40, 80 and 200 µg/ml for 3, 6, 12 and 24 h did not showed statistically significant change in cell viability. Sidh Makardhwaj exposure for 24 hours at maximum dose (200 µg/ml) showed 96.2 ± 5.0 % of cell viability in HepG2 cells and 101.9 ± 5.9 % of cell viability in HEK cells.

- The exposure of HepG2 and HEK cell with Arogyavardhini vati at 100, 200, 400 and 800 µg/ml for 3, 6, 12 and 24 h did not showed statistically significant change in cell viability. Arogyavardhini vati exposure for 24 hours at 100 µg/ml showed 110.2 ± 8.2 % of cell viability in HepG2 cell and 106.0 ± 8.1 % of cell viability in HEK cells.

- Exposure of HepG2 and HEK cell with Ras Sindur at doses of 25, 50, 100 and 200 µg/ml for 3, 6, 12 and 24 h did not showed significant change in cell viability. Ras
Sindur exposure for 24 hours at maximum dose (200 µg/ml) showed 101.5 ± 4.1 % of cell viability in HepG2 cells and 98.4 ± 3.3 % of cell viability in HEK cells.

- Exposure of HepG2 cell with Kajjali at doses of 50, 100, 200 and 400 µg/ml for 3, 6, 12 and 24 h did not showed significant change in cell viability. Kajjali exposure for 24 hours at maximum dose (200 µg/ml) showed 97.8 ± 6.1 % of cell viability in HepG2 cells and 94.6 ± 3.7 % of cell viability in HEK cells.

Thus, the results of the present cell culture study shows that Sidh Makardhwaj, Arogyavardhini vati, Ras Sindur and kajjali are non toxic to HepG2 and HEK cell at the doses upto eight times of therapeutic dose. Mercury, gold and mixture of mercury and gold solution caused significant decrease in cell viability in dose dependent manner. The composition of Sidh Makardhwaj is gold, mercury and sulphur in a specified ratio (1:8:24), Arogyavardhini vati is mercury (1 of 44 part), Ras Sindur is mercury and sulphur (1:6) and Kajjali is mercury and sulphur (1:6). When HepG2 and HEK cells were exposed with these mercury based Ayurvedic formulations in varying concentrations, there was no significant decrease in cell viability. Hence, it can also be concluded from the present study that Ayurvedic processing (Sodhana and Marana) during the preparation of these formulations plays an important role in detoxification of heavy metals present in these formulation. The results support the Ayurvedic literature that the processed mercury along with sulphur is converted to mercury sulphide and in low dose shows very good therapeutic activities without producing toxic effects in the human subjects.
14. Conclusions summarizing the achievements and indication of scope for future work.

Ayurveda is the traditional medicinal system of India originated approximately 5000 years ago. It recommends the use of plant based medicines as well as mineral based medicines including mercury, arsenic, lead, copper and gold for treating assorted disease conditions (Saper et al., 2008, Chaudhary, 2011). The use of these metals in Ayurvedic medicines is done after a rigorous process of purification (shodhana) and converting the metal into compounds (marana) (Sarkar, and Chaudhary, 2010). However, recently some metallic preparations used in Indian system of medicine are suspected to be harmful, causing hepatic, renal and neurotoxicity and many other side effects (Upadhyay et al., 2008). Recent reports on the presence of heavy metals in Ayurvedic preparations have raised much concern and controversy (Saper et al., 2004). On contrary, mercury along with sulphur (mercury sulphide) is one of the ingredients of many Ayurvedic medicines. Ayurvedic experts have reported that approximately 20% of the Ayurvedic formulations contain mercury sulphide as an ingredient (Ayurvedic Formulary of India, 2005; Gogtay et al., 2002). As per the classic Ayurvedic text, the processed mercury along with sulphur is converted to mercury sulphide and in low dose shows very good therapeutic activities without producing any toxic effects in the human subjects (Nishteswar and Vidyanath, 2005). Therefore, heavy metals content in metal based Ayurvedic preparation can still be thousand folds higher (Gogtay, 2002; Saper et al., 2008; Kappor, 2010).

A study by Chaudhary et al. (2011) showed that these bhasma are in nanometer dimension which may be considered as nanomedicine and free from toxicity in therapeutic doses (Chaudhary, 2011). Therefore, the present study was designed to evaluate the safety of Sidh Makardhwaj and Arogyavardhini vati, a mercury based Ayurvedic formulation in in vitro, in vivo and clinical study. Pilot prospective cohort clinical study was designed based on the safety data of cell culture and experimental animal study to evaluate the safety and efficacy
of Sidh Mkardhwaj in rheumatoid arthritis patients and Arogyavardhini vati in dyslipidemia patients.

The results of cell culture study shows that Sidh Makardhwaj, Arogyavardhini vati, Ras Sindoor and kajjali are non toxic to HepG2 and HEK cell at the doses up to eight times of therapeutic dose. In animal study, Sidh Makardhwaj upto 5 times and Arogyavardhini vati upto 10 times the equivalent dose administered to rats for 28 days did not show any toxicological effects on brain, liver and kidney.

The findings of clinical study suggest that Sidh Makardhwaj and Arogyavardhini vati is safe and effective. Hence, present Ayurvedic treatments (Ashwagandha powder and Sidh Makardhwaj) and (Arjuna powder and Arogyavardhini) can thus potentially be used for the treatment of rheumatoid arthritis and dyslipidemia patients respectively.

So, from the results of present study, it can be concluded that Ayurvedic processing (Sodhana and Marana) during the preparation of these formulations plays an important role in detoxification of heavy metals present in these formulation. The results support the Ayurvedic literature that the processed mercury along with sulphur is converted to mercury sulphide and in low dose shows very good therapeutic activities without producing toxic effects in the human subjects.

However, the safety of Arogyavardhini vati and Sidh Makardhwaja should be evaluated after chronic (90 days) treatment on rats. This will further strengthen the safety profile and corroborate with these clinical situations where long term treatments are given.

15. Procurement/usage of Equipment: NA

16. Manuscript for Publication (300 words for possible publication in Council’s Bulletin): Published articles attached
Name and signature with date

1. __________________________
   (Principal Investigator)

2. __________________________
   (Co-Investigator)

3. __________________________
   (Co-Investigator)

4. __________________________
   (Co-Investigator)