

PREPARATION, STANDARDIZATION AND EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ARKA LAVANA

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ABSTRACT: The liver is a vital organ of the human body. It is responsible for protecting the body from harmful substances and the maintenance of good health. In last few years, various types of liver disorders, such as drug-induced liver disease, hepatomegaly, fatty liver disease, liver cirrhosis, autoimmune hepatitis, chronic viral hepatitis, autoimmune hepatitis, etc. are become a people's health problem. Recently numbers of ayurvedic polyherbal and herbomineral formulations are available commercially and physician preferring these formulations for the treatment and management of various liver diseases as modern medical treatment available for liver diseases produces systematic toxicity. In this study a herbomineral formulation "Arka Lavana" was prepared in the laboratory by traditional method using fresh leaves of arka (*Calotropis procera*) and saindhav lavana and experimentally evaluated against hepatotoxicity induced by dexamethasone and alcohol. It is traditionally used in the treatment of liver and spleen anomalies and ascites etc. The study was evaluated by using 60 mg/kg 90 mg/kg and 120 mg/kg of arka lavana and standard silymarin at a dose of 100 mg/kg. The morphological, histopathological and biochemical parameters were evaluated on entry date and at the end of study. The results of the study indicate that the herbomineral formulation produces significant improvement in liver anomalies and the effect was dose-dependent manner.

Keywords:

Arka lavana, Hepatotoxicity, Dexamethasone, Silymarin, Bilirubin, SGPT and SGOT

INTRODUCTION: In the world, liver disease is a major public health issue and contributed markedly to the world mortality load ¹. Globally, approximately 20 lakh deaths found every year due to unhealthy liver condition ². In last few years, liver disorders, such as non-alcoholic liver disease, alcoholic liver disease, hepatomegaly, fatty liver disease, etc become a people's health problem and the main causes are drinking alcohol, viral infections, drug-induced, hereditary diseases, autoimmune diseases, etc ³.

Liver diseases induced by alcohol like alcoholic fatty liver, acute alcoholic hepatitis, and alcoholic cirrhosis are most common in the western world ⁴. Also, the occurrence of non-alcoholic liver diseases worldwide is high, and about 20% people of global population are affected by non-alcoholic fatty liver diseases ⁵. Recently, physicians are hesitating to prescribe allopathic medicines for long term treatment of liver disorders due to systemic toxicity induced by these medicines. But due to minimal side effects, ayurvedic medicines are more preferred for the treatment of liver anomalies over allopathic medicines ⁶. Further, scientific research also confirmed the effectiveness of ayurvedic medicines designed from plant origin or herbomineral origin in various liver disease and these preparations are recommended as primary or adjunctive herbal treatment for liver disease ⁷.

Near about 50% of Indian population depend on ayurvedic medicines for liver diseases and for the treatment of liver diseases more than three hundred herbomineral preparations are available in the Indian

system of medicines⁶. Arka lavana (AL) is one of herbomineral formulation *i.e.* described in lavana kalpana of ayurvedic text and it is mainly indicated for ykriptheeha rog (liver & spleen disease). It is traditionally used in the treatment of liver and spleen anomalies and also used in atisara (diarrhea), udar rog (ascites), *etc.*⁸ Arka lavana was traditionally prepared by using the fresh leaves of arka (*Calotropis procera*) & saindhav lavana⁹.

MATERIALS AND METHODS:

Collection of plant material: Leaves of *Calotropis procera* were collected during the month of November 2018 from Rajiv Gandhi South Campus, Mirzapur, Uttar Pradesh, India. The collected plant material was identified in Department of Botany, Faculty of Science, Banaras Hindu University, Varanasi, and the specimen voucher no is - Asclepia.2109/1 and saindhav lavana was collected from Dinanath Gola market, Varanasi, Uttar Pradesh, India.

Ingredients of Arka Lavana:

- Arka leaf
- Saindhav lavana
- Cotton cloth
- Multanimitti

Preparation of Arka Lavana: Arka lavana was prepared as per the literature available in the classical textbook Bhaishjya Yogratnavali¹⁰. At first a dried and cleaned sarav was taken and fresh leaves of arka (*C. procera*) were collected. The collected arka leaves were placed in a sandwich pattern with saindhav lavana. The pattern was maintained such a way that upper and lower layers were arka leaves. Another sarav was placed over it and sealed with seven-layer of mud (multani mitti) smeared cloth and after that it was ignited for certain time and waited for self-cooling. After cooling the joining portion was scraped out with knife and the inner material (the salt along with leaves) was collected in triturating mortar-pestle and made into fine powder. The formulation was preserved in an airtight container for further study and one commercial sample was purchased for comparative study.

Organoleptic Study: The organoleptic parameters like taste, odor, color and appearance were studied for both laboratory and marketed sample¹¹.

Physico-chemical and Pharmaceutical Standardization: The prepared arka lavana was standardized by evaluating various quality control parameters of the formulation, and for comparative study one commercial sample was procured. The physicochemical parameters like ash value, loss on drying, pH, water-soluble extractive value, fluorescence analysis¹² and pharmaceutical parameters like bulk density, tapped density, Hausner's ratio, compressibility and angle of repose were studied¹³.

Pharmacological Evaluation:

Animals:

Experimental Animals: Male albino rats of either sex weighing between 150-200 g were used in this study. Animals were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Animals were housed in large polypropylene cages in a temperature-controlled room (25 ± 20 °C). Humidity (55 ± 10%) was maintained properly and 12 h light and 12 h dark cycles were also followed. Animals were provided with standardized pelleted feed (Amrut Pvt. Ltd.) and clean drinking water. Rats were acclimatized to the standard laboratory condition for 15 days before using for experiments. The principle of laboratory animals cares guidelines (NIH Publication number 85-23, revised 1923) was followed. The study has got clearance from the Institutional Animal Ethical Committee (IAEC) for the purpose to

control and supervision of experiments on animals and the Animal Ethical Committee approval no. is Dean/2019/CAEC/1196. The animals fasted overnight before the experiment.

Dose Calculation: The human dose of arka lavana has been reported as 250 to 500 mg^{14, 15}. The highest dose has been considered for the experimental purpose. The animal dose of arka lavana was calculated using an approved dose conversion guide. The dose of 60 mg/kg (lowest dose), 90 mg/kg (middle dose) and 120 mg/kg (high dose) was estimated for as the animal dose based on body surface area ratio¹⁶.

Preparation of Dosage Form: Accurately weighed (against each subjected body weight) arka lavana were suspended with 0.5% CMC, for oral route of administration to test group. Silymarin was also suspended with 0.5% CMC for the oral route.

Dexamethasone was injected from intraperitoneal route. Ethanol 40% v/v (60% water and 40% alcohol) was given by oral route administration.

Chemicals and Reagents Used:

Drugs: Silymarin (Micro labs limited) and Arka lavana.

Hepatotoxic Agents: Dexamethasone and Ethanol.

Reagents Used:

- SGOT (ALT) Kit (Arrkay Healthcare Pvt. Ltd., Surat, India)
- SGPT (AST) Kit (Arrkay Healthcare Pvt. Ltd., Surat, India)
- Bilurubin Kit (Coral Clinical System Pvt Ltd., Uttarakhand, India)

Experimental Studies:

1. Anti-hepatotoxicity study induced by dexamethasone
2. Anti- hepatotoxicity study induced by Ethanol

Experimental Procedure:

Dexamethasone Induced Hepatotoxicity Model:

Animal Grouping and Drug Treatment: Fatty liver in rats was induced by oral administration of a single dose 8 mg/kg i. p. of dexamethasone and silymarin (100 mg/kg) was given as a standard drug^{17, 18}. The animals were grouped into six groups with 5 animals in each group.

Group I (Normal Control): Animals received 0.5% CMC (P.O.) daily for 7 days.

Group II (Negative Control): Animals received 0.5% CMC (P.O.) daily for 7 days and on 4th day, dexamethasone 8 mg/kg was given intraperitoneal.

Group III (Positive Control): Animals received the standard drug, silymarin once a day for 7 days and on the 4th day, dexamethasone 8 mg/kg was given intraperitoneal.

Group IV: Animals received arka lavana (60 mg/kg/day P.O.) once a day for 7 days and on the 4th day, dexamethasone 8 mg/kg was given intraperitoneal.

Group V: Animals received arka lavana (90 mg/kg/day P.O.) once a day for 7 days and on the 4th day, dexamethasone 8 mg/kg was given intraperitoneal.

Group VI: Animals received arka lavana (120 mg/kg/day P.O.) once a day for 7 days and on the 4th day, dexamethasone 8 mg/kg was given intraperitoneal.

On the 8th day, under mild anesthesia (diethyl ether) the blood sample was collected from retro-orbital sinus puncture (using capillary tubes) and cardiac puncture in a heparinized 1 ml tuberculin syringe. Later, rats were sacrificed at the end of study by cervical dislocation under mild ether anesthesia and the liver was separated and stored in 10% formalin solution for further estimation¹⁹.

Ethanol Induced Hepatotoxicity Model:

Animal Grouping and Drug Treatment: Fatty liver in rats was induced by oral administration of 40% ethanol and silymarin (100 mg/kg) was given as a standard drug. The animals were divided into six groups of five animals in each group and received the following regime of treatment ²⁰.

Group I (Normal Control): Animals received 0.5% CMC (P.O.) daily for 21 days.

Group II (Negative Control): Animals of this group received ethanol (40% v/v) 20 ml/kg (P.O.) daily for 21 days.

Group III (Positive Control): Animals of this group received silymarin (100 mg/kg P.O.) and ethanol (40% v/v) 20 ml/kg was given orally once a day for 21 days.

Group IV: Animals received arka lavana (60 mg/kg/day P.O.) once a day for 21 days and ethanol (40% v/v) 20 ml/kg was given orally.

Group V: Animals received arka lavana (90 mg/kg/day P.O.) once a day for 21 days and ethanol (40% v/v) 20 ml/kg was given orally.

Group VI: Animals received arka lavana (120 mg/kg/day P.O.) once a day for 21 days and ethanol (40% v/v) 20 ml/kg was given orally.

Biochemical Studies: The collected blood sample was centrifuged (using REMI, R-4C, Laboratory centrifuge) immediately at 2000 rpm for 5 min., when the serum clearly separated out. The serum was analyzed according to manual given with kit by the manufacture for SGOT, SGPT, and total bilirubin levels by using respective diagnostic kits ²¹.

Histopathological Studies: Liver samples collected from the animals were fixed with 10% formalin solution immediately for 72 h. The liver sample were washed with tap water after removing from formalin solution. After that liver samples were trimmed using sharp stainless steel blade. Trimmed tissue were dipped in 100% acetone, acetone: benzene (1:1) and benzene (100%) respectively for 2 h in each solvent. After that tissues were removed from benzene (100%) and dipped into melted wax and kept into incubator for 1 h. After that tissue were taken out from wax and dipped into melted wax (fresh) and kept into desiccators for another 1 h. Sections of blocks were cut using microtome and section of tissue were fixed into mixture of egg albumin, glycerin and phenol smeared slides. Sections were heat-fixed and dipped into xylene to remove wax. After removal of wax, tissue were stained using staining reagent haematoxylin and eosin then mounted with glycerin on slides. The photographs were taken at 40x with the help of compound microscope (Olympus, CH20IBIMF) ²².

Statistical Analysis: The result of various studies were expressed as Mean \pm SD and analyzed using one way ANOVA followed by Student's t-test using software SYSTAT 7.0, to find out the level of significance. Data were considered statistically at the minimum level of $P < 0.05$.

RESULTS:

Preparation of Arka Lavana:



FIG. 1: ARKA LEAVES



FIG. 2: SAINDHAV LAVANA



FIG. 3: SANDWICH OF ARKA LEAVES AND SAINDHAV LAVANA



FIG. 4: PREPARED ARKA LAVANA



FIG. 5: POWDERED ARKA LAVANA

Value are expressed as Mean-+SD (n = 5). Statical comparison was analysed by one way ANOVA followed by Dunnett's test where, * represents significant at $p < 0.05$, ** represent at $p < 0.01$, *** represent significant at $p < 0.001$, ns = non-significant. All values are compared with normal control

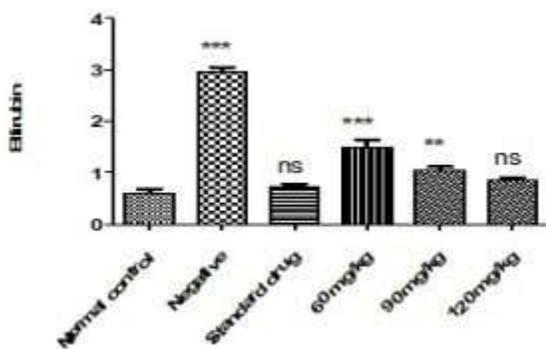


FIG. 6: EFFECT OF ARKA LAVANA ON SERUM SGPT LEVEL (DEXAMETHASONE INDUCED)

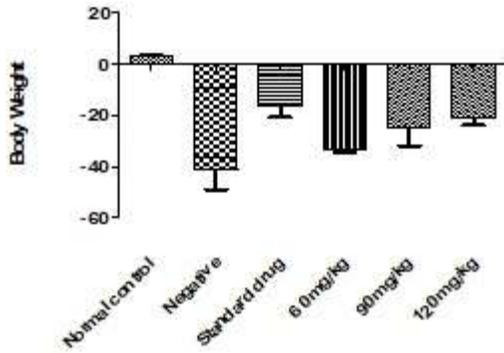


FIG. 7: EFFECT OF ARKA LAVANA ON SERUM SGOT LEVEL (DEXAMETHASONE INDUCED)

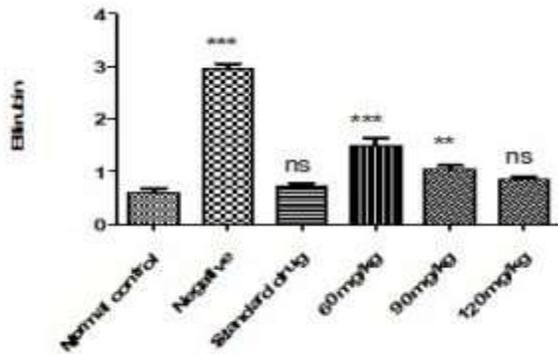


FIG. 8: EFFECT OF ARKA LAVANA ON SERUM BILIRUBIN LEVEL (DEXAMETHASONE INDUCED)

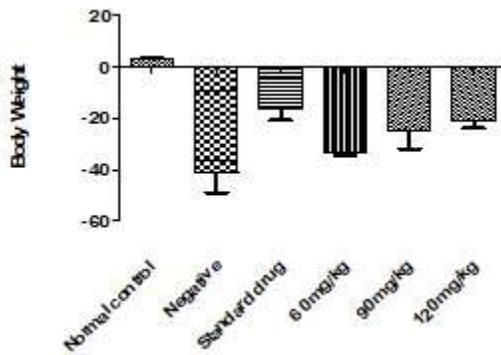


FIG. 9: EFFECT OF ARKA LAVANA OF BODY WEIGHT (DEXAMETHASONE INDUCED)

Gross Pathology:



FIG. 10: OBSERVATION BY NAKED EYE OF LIVER OF RATS IN DEXAMETHASONE INDUCED EXPERIMENTAL MODEL. (A) NORMAL CONTROL GROUP. (B) DEXAMETHASONE TREATED GROUP (C) DEXAMETHASONE + SILYMARIN TREATED GROUP. (D) DEXAMETHASONE + AL (60 mg/kg). (E) DEXAMETHASONE + AL (90 mg/kg). (F) DEXAMETHASONE + AL (120 mg/kg)

Histopathological Studies:

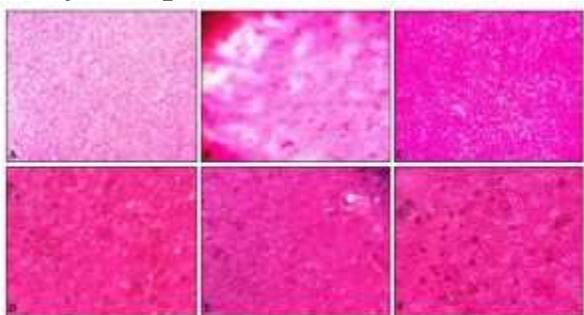


FIG. 11: HISTOPATHOLOGICAL EXAMINATION OF EOSIN-STAINED LIVER SECTION HISTOPATHOLOGICAL EXAMINATION OF EOSIN-STAINED LIVER SECTION OF NORMAL AND EXPERIMENTAL RATS (A) NORMAL CONTROL GROUP. (B) DEXAMETHASONE TREATED GROUP (C) DEXAMETHASONE + SILYMARIN TREATED GROUP. (C) DEXAMETHASONE + AL (60 MG/KG). (D) DEXAMETHASONE + AL (90 MG/KG). (E) DEXAMETHASONE + AL (120 MG/KG)

Effect of Silymarin, Arka lavana (AL) on Ethanol Induced Hepatotoxicity in Rat:

Value are expressed as Mean \pm SD (n=5). Statical comparison was analysed by one way ANOVA followed by Dunnett's test where, * represents significant at $p < 0.05$, ** represent at $p < 0.01$, *** represent significant at $p < 0.001$, ns = non-significant. All values are compared with normal control

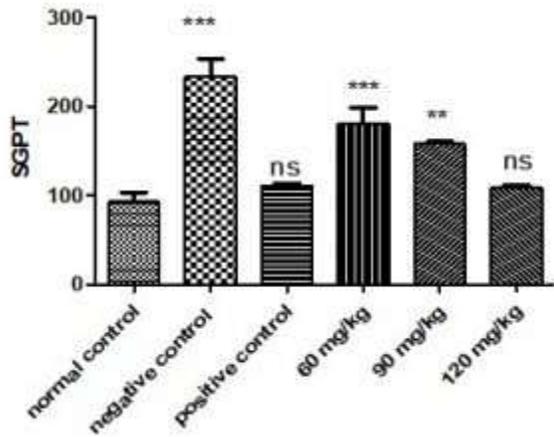


FIG. 12: EFFECT OF ARKA LAVANA ON SERUM SGPT LEVEL (ETHANOL INDUCED)

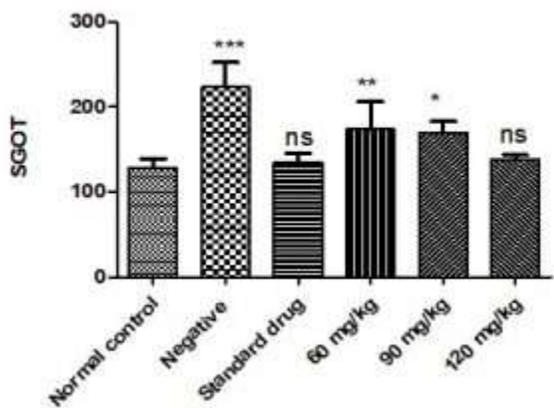


FIG. 13: EFFECT OF ARKA LAVANA ON SERUM SGOT LEVEL (ETHANOL INDUCED)

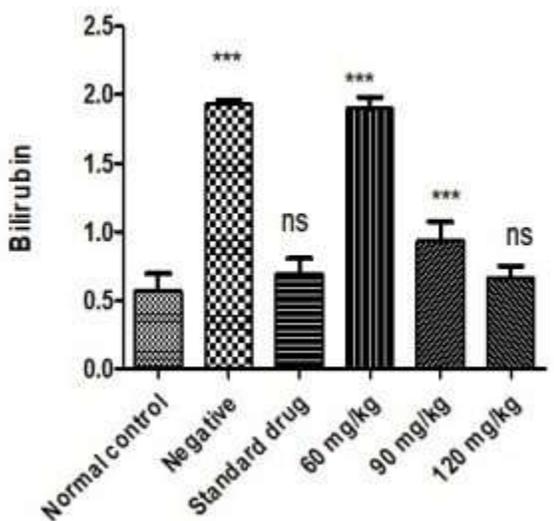


FIG. 14: EFFECT OF ARKA LAVANA ON SERUM BILIRUBIN LEVEL (ETHANOL INDUCED)

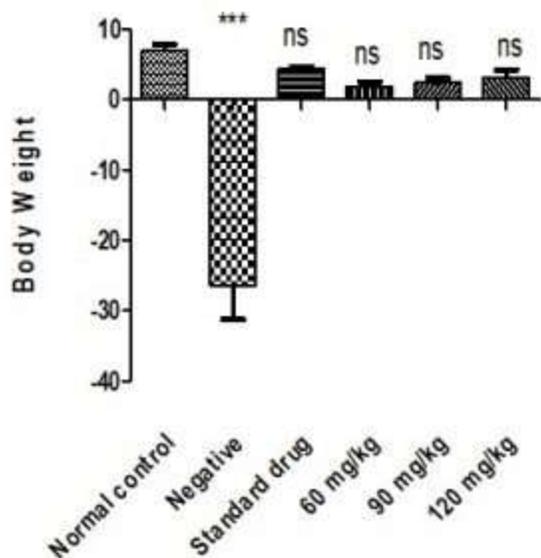


FIG. 15: EFFECT OF ARKA LAVANA OF BODY WEIGHT (ETHANOL INDUCED)

Gross Pathology:

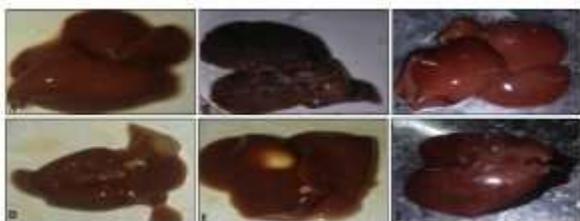


FIG. 16: NAKED EYE OBSERVATION OF LIVER OF RATS IN ETHANOL INDUCED MODEL (A) NORMAL CONTROL GROUP (B) ETHANOL TREATED GROUP (C) ETHANOL + SILYMARIN TREATED GROUP. (D) ETHANOL + AL (60 mg/kg). (E) ETHANOL+ AL (90 mg/kg). (F) ETHANOL + AL (120 mg/kg)

Histopathological Studies:

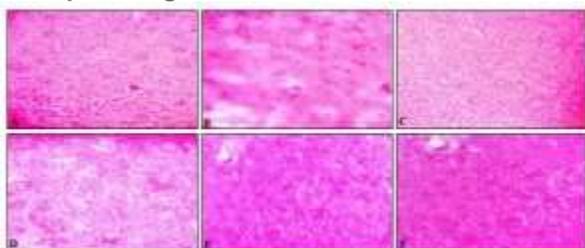


FIG. 17: HISTOPATHOLOGICAL EXAMINATION OF EOSIN-STAINED LIVER SECTION HISTOPATHOLOGICAL EXAMINATION OF (HE) HEMATOXYLENE AND EOSIN-STAINED LIVER SECTION OF NORMAL AND EXPERIMENTAL RATS (A) NORMAL CONTROL GROUP (B) ETHANOL TREATED GROUP (C) ETHANOL + SILYMARIN TREATED GROUP. (D) ETHANOL + AL (60 mg/kg). (E) ETHANOL+ AL (90 mg/kg). (F) ETHANOL + AL (120 mg/kg)

DISCUSSION: In the Indian system of medicines, herbomineral formulations are effective as like other formulations. Herbomineral formulations are not only used for the treatment of disease but also used for the maintenance of good health. These formulations act through holistic approach with fewer side effects ²³.

In this study a similar approaches were made to standardized and explore the biological activity of a classical ayurvedic formulation *i.e.* arka lavana in experimental models.

Arka lavana is the type of lavanakalpana *i.e.* herbomineral formulation consist of arka leaf (*C. procera*) and saindhava lavana (rock salt). This formulation traditionally recommended for various diseases like abdominal disease, splenomegaly, and constipation, *etc.*⁸. Arka lavana was prepared as per classical text using authenticated plant and mineral raw drugs and the prepared herbomineral formulation was standardized by evaluating organoleptic parameters, physicochemical parameters and pharmaceutical parameters¹¹. The physicochemical parameters like ash value, loss on drying, pH, water-soluble extractive value, fluorescence analysis¹² and pharmaceutical parameters like bulk density, tapped density, Hausner's ratio, compressibility and angle of repose were studied¹³.

Further, for comparative studies a commercial sample was procured from the VHCA, Ayurveda. The organoleptic study revealed that Arka lavana of laboratory sample was roughly powdered blackish-brown in color while commercial sample was coarsely powdered brown. The evaluated organoleptic parameters are mentioned in **Table 1**. Physico-chemical evaluation of both samples of arka lavana were done and parameters like loss on drying, ash value, pH, and water-soluble extractive value were evaluated and mentioned in **Table 2**¹².

Organoleptic screening and physicochemical studies were made to standardize the herbomineral formulation, which will be helpful to assess the formulation and protection from adulteration, and substitution of the product²⁴. The pharmaceutical parameters like bulk density, tapped density, Hausner's ratio, compressibility and angle of repose were studied to represent the flowability and compressibility of arka lavana¹³. The study showed that both the sample have close resemblance in pharmaceutical and physicochemical data. Standardization of herbomineral formulation based on pharmaceutical and physicochemical aspects are highly essential to justify its therapeutic activity experimentally and acceptability among the physicians²⁵.

The prepared laboratory sample was evaluated for hepatoprotective activity in rat model. The study was evaluated in dexamethasone induced hepato-toxicity model and ethanol-induced hepatotoxicity model. In this study, both dexamethasone treated group and ethanol-treated group showed significant reduction in body weight and gained relative organ weight. The level of SGPT, SGOT, and serum bilirubin were markedly increased *i.e.* proved by gross pathology and histopathology. This evidence confirmed hepatotoxicity in animals treated with dexamethasone and ethanol. Ethanol induces hepatotoxicity by decreasing level of hepatic glutathione and increasing the level of ALT, AS and gamma-glutamyl transferase²⁶.

Silymarin treated group animals in both dexamethasone induced and ethanol-induced study showed significant change in body weight reduction in comparison to negative control group and non-significant change to normal control group. Also the study showed significant change in serum level SGPT, SGOT and bilirubin to negative control group, whereas this group animals showed non-significant change in serum level SGPT, SGOT and bilirubin with respect to normal control group of animals.

Test-I group of animals' in dexamethasone induced study showed non-significantly change in bodyweight reduction to negative control group and significantly change with respect to normal control group whereas, in ethanol-induced study, test 1 group animals showed significant change in body weight growth and relative organ weight to negative control group and non-significantly change to normal control group. But the level of serum SGPT, SGOT, and bilirubin in both the studies showed non significantly change with respect to negative control group and significantly change with respect to normal and positive control group.

Test-II group animals of both studies showed similar types of results as shown by test 1 group animals. Test-III group animals in both dexamethasone induced model and alcohol-induced model showed a non-significant change in bodyweight reduction with respect to positive control group and significant change with respect to negative control group, also both the study showed a non significant change in level of serum SGPT, SGOT, and bilirubin with respect to both normal control and positive control group and significant change with respect to negative control group.

On the day of terminal scarification of dexamethasone induced model and ethanol-induced model group animals, the gross pathological study showed the normal treated group had no abnormality in liver; the negative group showed increase in liver weight with fibrosis and nodes; standard group showed similar observation as shown in normal group animal.; Test-I groups showed the fibrosis with increase in liver weight compare to normal group, but less than negative group; Test-II group showed further reduction in liver weight and fibrosis comparative to Test-I group and Test-III group showed similar observations as standard and normal treated group. These observations show that arka lavana has dose-dependent effect on hepatotoxicity.

Histopathological studies of dexamethasone induced model and ethanol-induced model, the rat liver tissues of control group showed normal hepatic cells with central vein. The negative group animals liver tissues showed severe hepatotoxicity in the form of several inflammations. Whereas the histopathological study of liver tissues showed that the pathological lesions caused by dexamethasone and ethanol were very minimal in groups treated with AL (120 mg/kg) (figure e) and liver tissue had normal hepatic cells in the animals treated with standard drug silymarin (figure c).

CONCLUSION: Arka lavana is one of herbomineral formulation which is traditionally used in the treatment of liver and spleen anomalies. It was traditionally prepared by using the fresh leaves of arka (*Calotropis procera*) & saindhav lavana. The prepared formulation was standardized by evaluating the various physicochemical and pharmaceutical parameters. The effectiveness of this formulation against liver anomalies was evaluated experimentally in dexamethasone induced and ethanol-induced hepatotoxicity rat model. This study was evaluated by using 60 mg/kg, 90 mg/ kg and 120 mg/kg doses of arka lavana and silymarin as standard drug. In this study biochemical parameters like SGOT, SGPT, total bilirubin, and body weight were measured to represent the effectiveness of the formulation. Gross pathology and histopathological of liver tissue were observed to confirm the effectiveness. The study showed arka lavana produces a dose-dependent effect and reduces the anomalies induced by dexamethasone and ethanol, similarly like silymarin. This study suggested that further study on arka lavana is necessary to consider it as a hepatoprotective agent.

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CONFLICTS OF INTEREST: Nil

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