IJPSR (2019), Volume 10, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 October 2019; received in revised form, 23 November 2019; accepted, 26 November 2019; published 01 December 2019

PREPARATION, STANDARDIZATION AND EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ARKA LAVANA

Jyoti Singh, A. K. Singh, A. K. Kushwaha, D. N. S. Gautam and Manmath K. Nandi *

Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi - 221005, Uttar Pradesh, India.

Keywords:

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

Correspondence to Author:
Dr. M. K. Nandi

Assistant Professor,
Department of Medicinal Chemistry, Faculty of Ayurveda,
IMS, BHU, Varanasi - 221005,
Uttar Pradesh, India.

E-mail: manmath.nandi@gmail.com

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.

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].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(12).5612-21

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(12).5612-21

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 medicines [6]. Arka lavana (AL) is one of herbomineral formulation *i.e.* described in lavana kalpana of ayurvedic text and it is mainly indicated for ykritpleeha 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 lavana [9].

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 Yogratnavali [10]. 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 sample [11].

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 analysis [12] and pharmaceutical parameters like bulk density, tapped density, Hausner's ratio, compressibility and angle of repose were studied [13].

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 ºC). 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 ratio [16].

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 (Arrkray Healthcare Pvt. Ltd., Surat, India)
- SGPT (AST) Kit (Arrkray 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 estimation [19].

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 group 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, $CH_20IBIMF$) [22].

Statistical Analysis: The result of various studies were expressed as Mean ± 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

TABLE 1: ORGANOLEPTIC STUDY

Test	Laboratory sample	Marketed sample
Colour	Black	Yellow
Taste	Salt	Salt
Odour	Pungent	Characteristic
Touch	Rough	Rough

TABLE 2: PHARMACEUTICAL STUDY

Test	Laboratory sample	Marketed sample
Bulk density	0.83	0.76
Tapped density	1.1	1.5
Haunser's ratio	1.33	1.45
Compressibility	24.54%	27.6%
Angle of repose	38^0	30^0

TABLE 3: PHYSICO-CHEMICAL STUDY

Test	Laboratory Sample	Marketed sample
Loss on drying	9.96 g	8.45 g
Total ash	94%	87%
Acid insoluble ash	53.6%	45.34%
Water soluble ash	88.4%	78.5%
Water soluble extractive value	83.78	58.6
pH	9.34	9.89

TABLE 4: FLUORESCENCE POWDER DRUG ANALYSIS OF LABORATORY AND MARKETED SAMPLES

Florescence + Reagent	Laboratory sample		Marketed sample	
	Florescence in day light	Florescence in (365) nm	Florescence in day light	Florescence in (365) nm
Powder as such	Black	NF	Yellowish-brown	NF
Powder + 1N NaOH	Med grey	Jade green	Dark smoky	Dark smoky
Powder+ 1 N NaOH in methanol	Blue-grey	Jade green	Brunt umber	Grey
Powder + 1 N HCl	Strom cloud	Mint green	Silver	Gold foil
Powder + 1 N HCl in methanol	Dark cycon	Kiwi green	Brunt sienna	Gold foil
Powder + 1 N HNO_3 in methanol	Strom cloud	Lime green	Light tan	Mint green
Powder + 1 N HNO_3	Cool grey	Kiwi green	Khaki	Dark yellow
Powder + I_2 (5%)	Warm grey	Black	Brunt umber	Vandyke brown
Powder+$FeCl_3$ (5%)	Mocha	Black	Brown	NF
Powder+ KOH	Dark grey	Forest green	Brown	Scarab green
Powder +NH_3(25%)	Silver grey	Kiwi green	Brunt umber	Olive green
Powder + acetic acid	Med grey	Bamboo color	Red	Peach

Hepatoprotective Activity of Standard Silymarin, Arka lavana (AL) on Dexamethasone Induced Hepatotoxicity in Rat:

Biochemical Studies:

TABLE 6: SERUM BIOCHEMICAL PARAMETERS:

Group	Treatment	SGPT (IU/L)	SGOT(IU/L)	**Bilirubin** (mg/100 ml)	Body weight change
I	Normal control	90.5 ± 8.96	129.720 ± 10.36	0.60 ± 0.19	3.20 ± 1.09
II	Negative control (Dexamethasone)	250.24 ± 12.3***	223.9 ± 29.34***	1.716 ± 0.17***	-41.0 ± 17.5***
III	Positive control (Silymarin)	95.08 ± 7.46ns	135.1 ± 11.20ns	0.726 ± 0.12ns	-16.400 ± 9.81*
IV	AL 60 mg/kg	203.67 ± 13.15***	174.9 ± 32.55**	1.49 ± 0.33***	-33.0 ± 3.39***
V	AL 90 mg/kg	154.67 ± 12.25***	173.7 ± 13.65**	1.051 ± 0.13**	-25.0 ± 15.16**
VI	AL 120 mg/kg	91.82 ± 10.67ns	140.52 ± 4.93ns	0.686 ± 0.139ns	-20.8 ± 6.72**

Value are expressed as Mean-+SD (n = 5). Statical comparison was analysed by one way ANOVA followed by Dunnnett'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

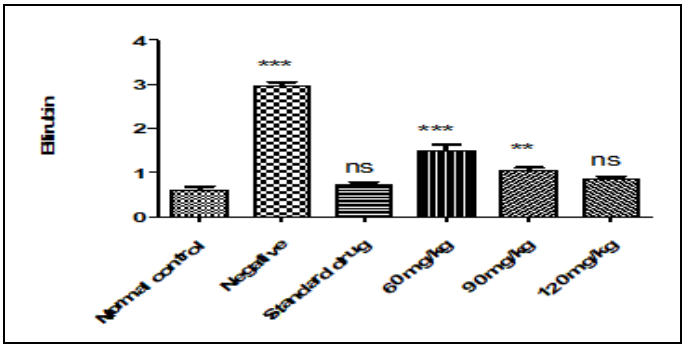

FIG. 6: EFFECT OF ARKA LAVANA ON SERUM SGPT LEVEL (DEXAMETHASONE INDUCED)

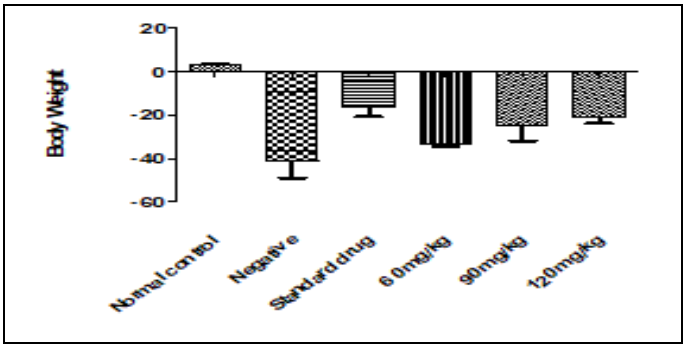

FIG. 7: EFFECT OF ARKA LAVANA ON SERUM SGOT LEVEL (DEXAMETHASONE INDUCED)

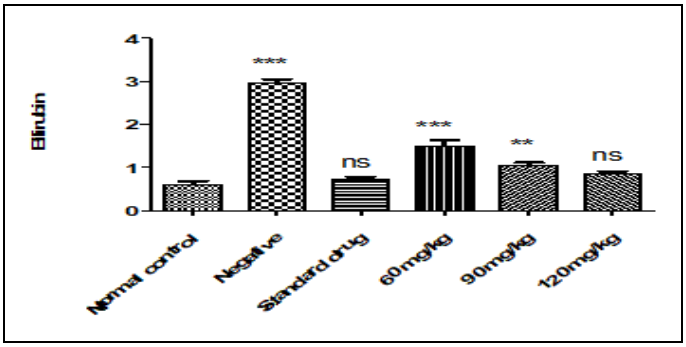

FIG. 8: EFFECT OF ARKA LAVANA ON SERUM BILIRUBIN LEVEL (DEXAMETHASONE INDUCED)

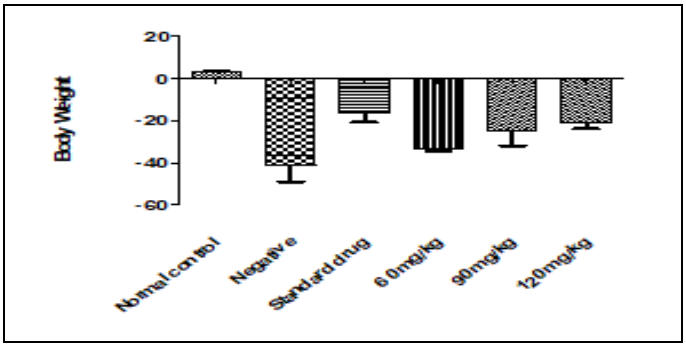

FIG. 9: EFFECT OF ARKA LAVANA OF BODY WEIGHT (DEXAMETHASONE INDUCED)

Gross Pathology:

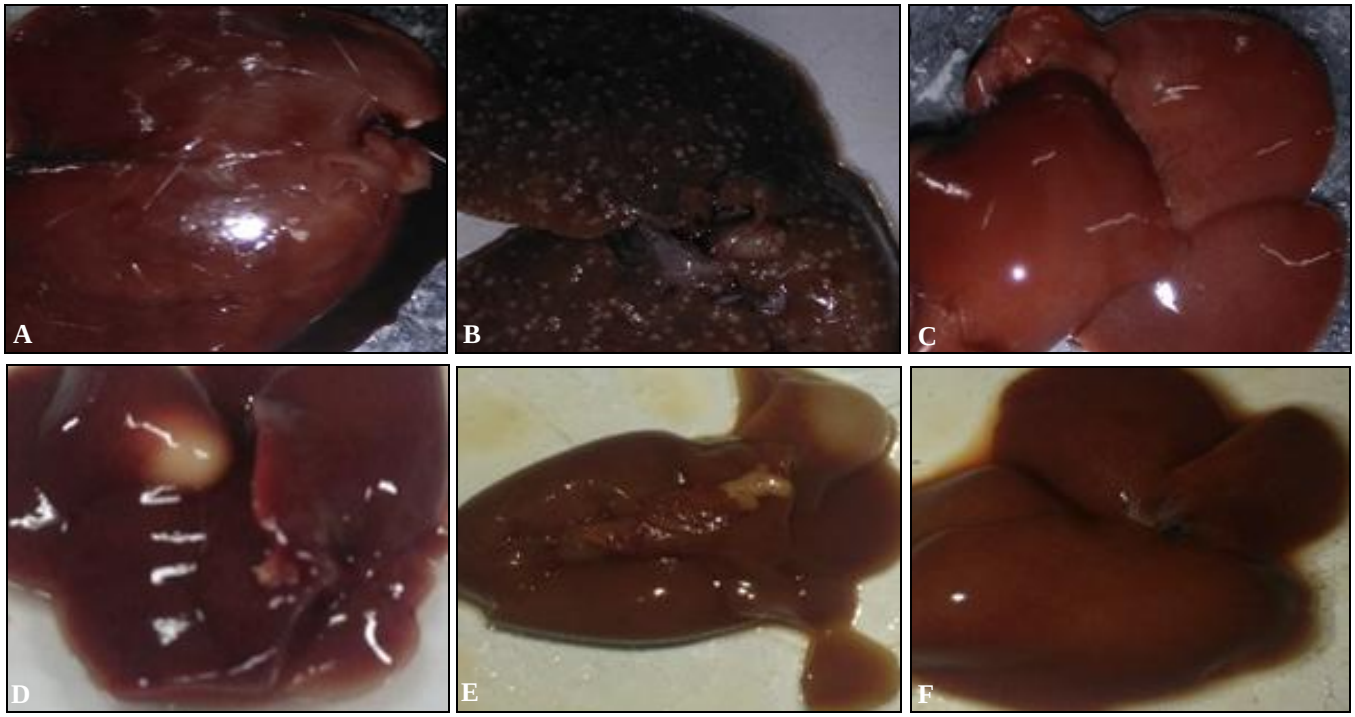

FIG. 10: OBSERVATION BY NAKED EYE OF LIVER OF RATS IN DEXAMETHASONE INDUCED EXPERIMENTAL MODEL. (A) NORMAL CONTROL GROUP. (B) DEXAMETHASONE TREATED GROUP (C) DEXAMETHASONE + SILYMARIN TREATED GROUP. (D) DEXAMETHASONE + AL (60 mg/kg). (E) DEXAMETHASONE + AL (90 mg/kg). (F) DEXAMETHASONE + AL (120 mg/kg)

Histopathological Studies:

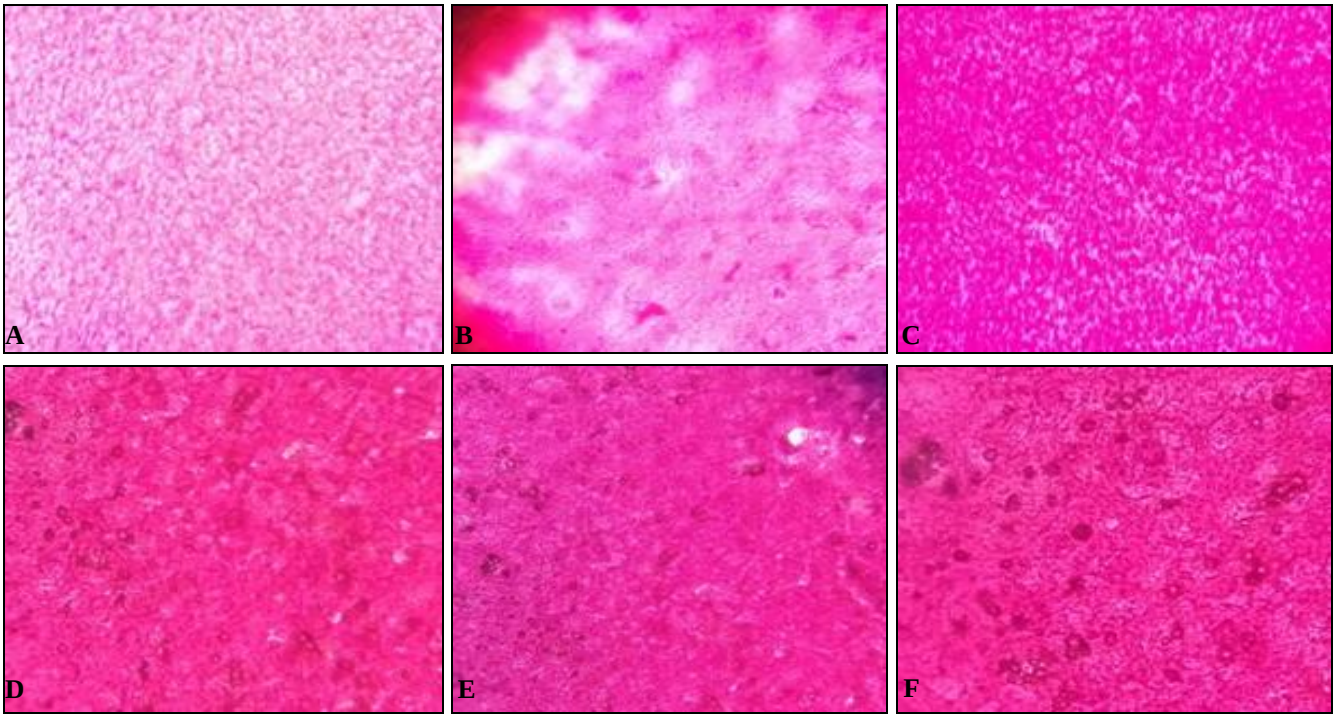

FIG. 11: HISTOPATHOLOGICAL EXAMINATION OF EOSIN-STAINED LIVER SECTION HISTOPATHOLOGICAL EXAMINATION OF EOSIN-STAINED LIVER SECTION OF NORMAL AND EXPERIMENTAL RATS (A) NORMAL CONTROL GROUP. (B) DEXAMETHASONE TREATED GROUP (C) DEXAMETHASONE + SILYMARIN TREATED GROUP. (C) DEXAMETHASONE + AL (60 MG/KG).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

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

TABLE 7: SERUM BIOCHEMICAL PARAMETERS

Group	Treatment	SGPT (IU/L)	SGOT (IU/L)	Bilirubin (mg/100 ml)	Body Weight change
I	Normal control	93.17 ± 10.42	128.72 ± 10.36	0.566 ± 0.129	7.00 ± 1.87
II	Negative control (Ethanol)	233.75 ± 20.04***	223.52 ± 28.34***	1.93 ± 0.031***	-26.4 ± 10.83***
III	Positive control (Silymarin)	110.72 ± 2.82ns	134.194 ± 11.20ns	0.69 ± 0.115ns	4.4 ± 0.54ns
IV	AL 60 mg/kg	180.17 ± 17.91***	173.93 ± 32.36**	1.90 ± 0.083***	1.9 ± 1.51ns
V	AL 90 mg/kg	158.02 ± 3.04***	170.37 ± 13.03*	0.93 ± 0.14***	2.4 ± 1.51ns
VI	AL 120 mg/kg	108.21 ± 3.23ns	138.52 ± 4.81ns	0.66 ± 0.082ns	3.200 ± 2.28ns

Value are expressed as Mean ± SD (n=5). Statical comparison was analysed by one way ANOVA followed by Dunnnett'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

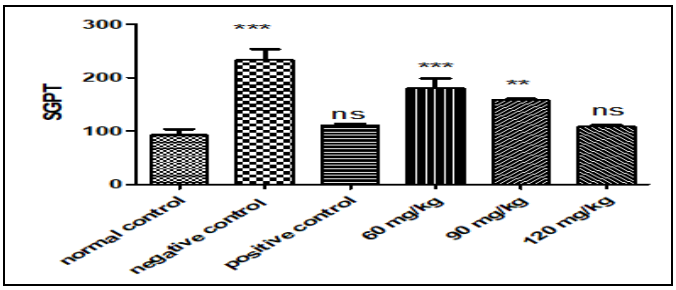

FIG. 12: EFFECT OF ARKA LAVANA ON SERUM SGPT LEVEL (ETHANOL INDUCED)

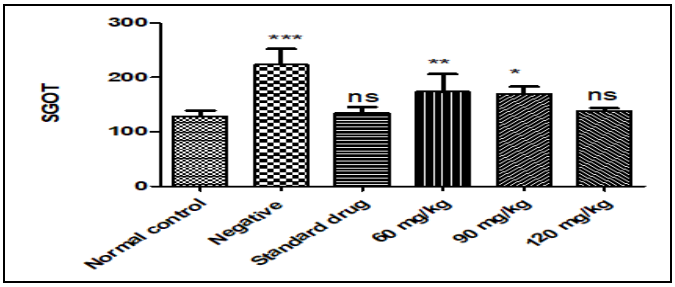

FIG. 13: EFFECT OF ARKA LAVANA ON SERUM SGOT LEVEL (ETHANOL INDUCED)

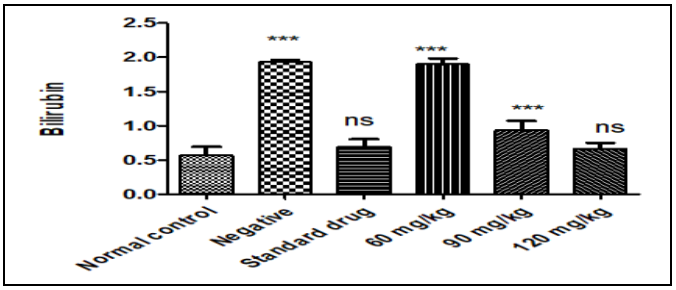

FIG. 14: EFFECT OF ARKA LAVANA ON SERUM BILIRUBIN LEVEL (ETHANOL INDUCED)

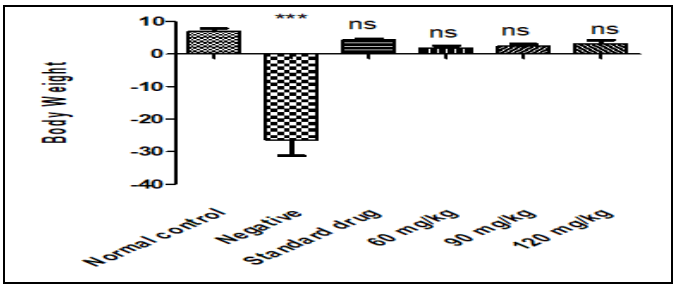

FIG. 15: EFFECT OF ARKA LAVANA OF BODY WEIGHT (ETHANOL INDUCED)

Gross Pathology:

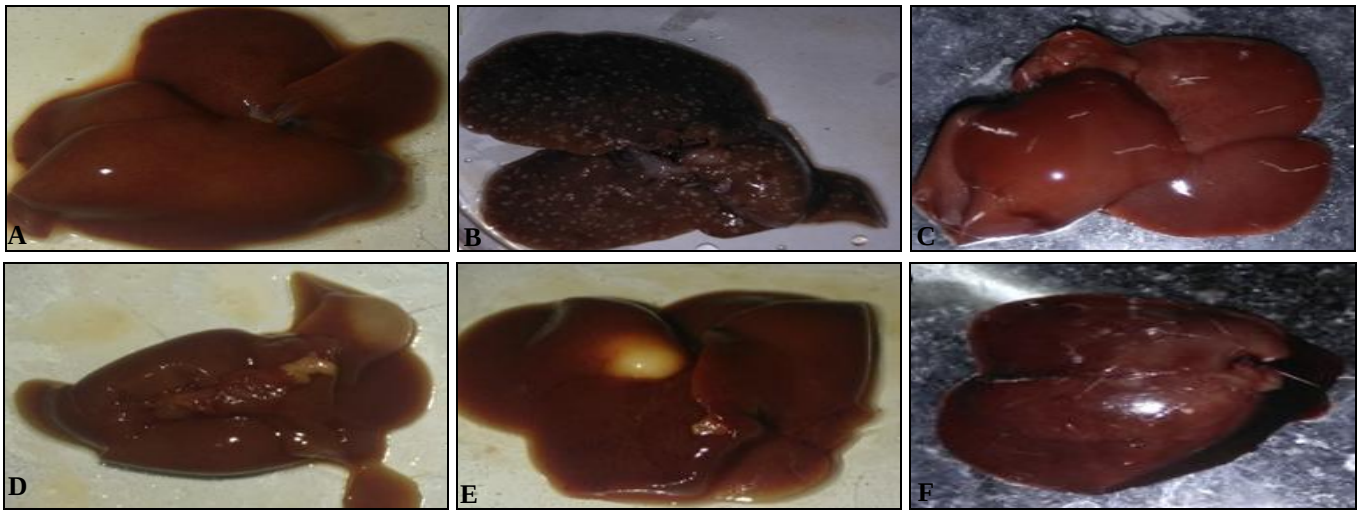

FIG. 16: NAKED EYE OBSERVATION OF LIVER OF RATS IN ETHANOL INDUCED MODEL (A) NORMAL CONTROL GROUP (B) ETHANOL TREATED GROUP (C) ETHANOL + SILYMARIN TREATED GROUP. (D) ETHANOL + AL (60 mg/kg). (E) ETHANOL+ AL (90 mg/kg). (F) ETHANOL + AL (120 mg/kg)

Histopathological Studies:

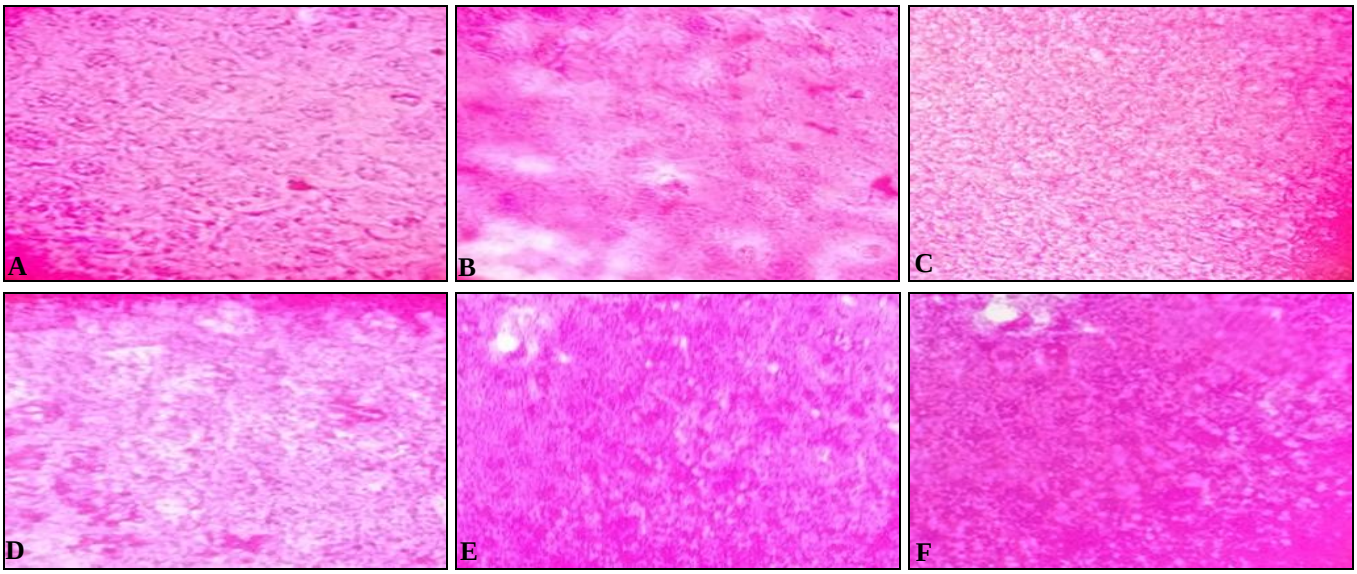

FIG. 17: HISTOPATHOLOGICAL EXAMINATION OF EOSIN-STAINED LIVER SECTION HISTOPATHOLOGICAL EXAMINATION OF (HE) HEMATOXYLENE AND EOSIN-STAINED LIVER SECTION OF NORMAL AND EXPERIMENTAL RATS (A) NORMAL CONTROL GROUP (B) ETHANOL TREATED GROUP (C) ETHANOL + SILYMARIN TREATED GROUP. (D) ETHANOL + AL (60 mg/kg). (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 parameters [11]. The physicochemical parameters like ash value, loss on drying, pH, water-soluble extractive value, fluorescence analysis [12] and pharmaceutical parameters like bulk density, tapped density, Hausner's ratio, compressibility and angle of repose were studied [13].

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 product [24]. 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 lavana [13]. 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 physicians [25].

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 transferase [26].

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.

ACKNOWLEDGEMENT: The authors are thankful to Banaras Hindu University, Varanasi, India for the help to perform the experiment.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Rehm J, Samokhvalov AV and Shield KD: Global burden of alcoholic liver diseases. J of Hepato 2013; 59(1): 160-8.
2. Asrani SK, Devarbhavi H, Eaton J and Kamath PS: Burden of liver diseases in the world. Journal of Hepatology 2019; 70(1): 161-71.
3. Shimizu Y: Liver in systemic diseases. World J Gastroenterol 2008; 14(26): 4111-19.
4. Walsh K and Alexander G: Alcoholic liver disease. Postgraduate Medical Journal 2000; 76 (895): 280-86.
5. Sattar N, Forrest E and Preiss D: Non-alcoholic fatty liver disease. BMJ 2014; 349: 4596
6. Panda AK, Bhuyan GC and Rao MM: Ayurvedic intervention for hepatobiliary disorders: current scenario and future prospect. Journal of Traditional Medicine and Clinical Naturopathy 2017; 6(1): 1-5.
7. Del Prete A, Scalera A, Ladevaia MD, Miranda A and Zulli C: Herbal product: benefits, limits and applications in chronic liver disease. Evidence based complementary and alternative medicine 2012; 1-19.
8. Sharma S: Rasa Tarangini, edition 6th. Motilal, Banarasidas, Varanasi, 2009; 351.
9. Devnathan R, Niraimathai KL, Karunanidhi M and Brindha P: Comparative evaluation of Arka lavana – An ayurvedic herbomineral formulation. International Journal of Pharma and Clinical Research 2013; 5(2): 37-42.
10. Mishra SN: Bhaishajya Ratnavali of Kaviraj Govind Das Sen Yakrita-plihadhikara, Reprint edition. Varanasi. Chaukhamba Surbharati Prakashan 2007; 41/30-39: 749-50.
11. Ahmed S and Hasan MM: Standardization of crude drugs: A precise review. World Journal of Pharmaceutical Research 2015; 4 (10): 155-74.
12. Lohar DR: Protocol for testing of Ayurvedic, Siddha and Unani medicines. Pharmacopoeial Laboratory for Indian Medicine, AYUSH. Ministry of Health and Family Welfare. Government of India, Ghaziabad 2011.
13. Shah RB, Tawakkul MA and Khan MA: Comparative evaluation of flow for pharmaceutical powders and granules. AAPS Pharm Sci Tech 2008; 9(1): 250-28.
14. Kishore SD and Hussain G: A review – on different yogas of arka. International Ayurvedic Medical Journal 2015; 3(5): 1474-82.
15. Rao PG: A test book of Bhaisjya Kalpana Vijnanam. Chaukhambha publications, edition 1st 2008; 178.
16. Nair AB and Jacob S: A simple practice guide for dose conversion between animals and human. Journal Of Basic and Clinical Pharmacy 2016; 7: 27-31.
17. Bhat GMN, Nayak N, Vinodraj K, Chandralekha N, Mathai P and Cherian J: Comparison of the efficacy of cardamom (*Elettaria cardamomum*) with pioglitazone on dexamethasone-induced hepatic steatosis, dyslipidemia, and hyperglycemia in albino rats. Journal Advanced Pharma Technology and Research 2015; 6(3): 136-140.
18. El-Sawy AEF, EL-Maddawy ZK and Ashoura NR: Role of silymarin in restoring the deleterious effects induced by dexamethasone in male rats. Alexandria Journal of Veterinary Sciences 2018; 59(2): 125-35.
19. Idoh K, Dosseh K, Kpatcha T, Agbonon A and Gbeassor M: Protective effect of Combretum hypopilinum diels: Root bark extract against CCl4-Induced hepatotoxicity in wistar rats. Pharmacognosy Research 2018; 10(3): 325-31.
20. Sharma A, Sangameswaran B, Jain V and Saluja MS: Hepatoprotective activity of Adina cordifolia against ethanol induce hepatotoxicity in rats. International Current Pharmaceutical Journal 2012; 1(9): 279-84.
21. Raju NJ and Sreekanth N: Investigation of hepatoprotective activity of *Ficus retusa* (Moraceae). International Journal of Research in Ayurveda and Pharmacy 2011; 2(1): 166-69.
22. Quaresma AB, Acampora AJD, Tramonte R, Farias DCD and Joly FS: Histologcal study of the liver and the biochemistry of the blood of Wistar rat following ligature right hepatic duct. Acta Cir Bras 2007; 22(1): 68-78.
23. Vakhariya RR, Talokar SS, Dhole AR and Magdum CS: Herbomineral formulation –a review. IJSRST 2015; 1(3): 161-69.
24. Madhav NVS, Upadhyaya K and Bisht A: Phytochemical screening and standardization of polyherbal formulation for dyslipidemia. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(3): 235-38.
25. Shinde JS, Khurde SS, Suchita L, Chavan SS and Hulmage SB: Need of polyherbal formulations and its standardization: a review. World Journal of Pharmacy and Pharmaceutical Sciences 2016; 5(11): 526-33.
26. Dixit N, Baboota S, Kohli K, Ahmad S and Ali J: Silymarin: A review of pharmacological aspects and bioavailability enhancement approaches. Indian Journal of Pharmacology 2007; 39(4): 172-79.

How to cite this article:
Singh J, Singh AK, Kushwaha AK, Gautam DNS and Nandi MK: Preparation, standardization and evaluation of hepatoprotective activity of arka lavana. Int J Pharm Sci & Res 2019; 10(12): 5612-21. doi: 10.13040/IJPSR.0975-8232.10(12).5612-21.