IJPSR (2022), Volume 13, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 April 2022; received in revised form, 26 May 2022; accepted, 16 June 2022; published 01 December 2022

EVALUATION OF ACUTE AND SUBCHRONIC TOXICITY OF POLYHERBAL FORMULATIONS OF SHREE DHANWANTRI HERBALS IN WISTAR RATS

Balbir Singh [2], Palvi Sharma [1], Ankita Rajput [1], Gurmeet Singh [3] and Saroj Arora [*1]

Department of Botanical and Environmental Sciences [1], Department of Pharmaceutical Sciences [2], Guru Nanak Dev University, Amritsar - 143005, Punjab, India.
Shree Dhanwantri Herbals [3], Amritsar - 143006, Punjab, India.

Keywords:

Toxicity, Shree dhanwantri herbals, Herbal formulations, Histopathology, OECD guidelines

Correspondence to Author:
Saroj Arora

Professor,
Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar - 143005, Punjab, India.

E-mail: sarojarora.gndu@gmail.com

ABSTRACT: Plants have been utilized as medicine since the ancient period, and they are at the basis of much of modern medicine. Plants provide the basis for many conventional medications and the majority of the few successful drugs. Herbal formulations are becoming increasingly popular nowadays. But in some cases, side effects of the herbal formulations have been reported. So, according to the AYUSH ministry, herbal formulations should also be evaluated for toxicity to determine their safe dose. This study aims to critically assess the scientific evidence for herbal formulations' acute and subchronic toxicity. Six herbal formulations of Shree Dhanwantri Herbals were tested for toxicity in rats. An acute and subchronic toxicity study was conducted on Wistar rats following OECD-423 and OECD-408 guidelines, respectively. No mortality or abnormal behavior was observed in the acute toxicity studies. In the sub-chronic toxicity study, testing formulations did not produce any significant changes in rats' behavior compared to the control group. Further, hematological and biochemical parameters were also found normal. Histopathological analysis revealed that treated animals' brain, lung, heart, kidney, liver, and spleen were not affected. A critical analysis of results points out that all herbal formulations under study produced no significant signs of toxicity.

INTRODUCTION: Ayurveda is one of the traditional medical sciences of India. It is based on preventing unnecessary suffering and living a long healthy life [1]. Ayurveda comprises the use of natural elements to get rid of the disease and promote a healthy lifestyle to prevent disease recurrence.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(12).5068-79

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

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(12).5068-79

According to World Health Organization, 80% of the world population still relies on traditional medicines for their health care [2]. In Ayurveda, single or multiple herbs (polyherbal) can beused for the treatment. For the vast majority of the world's population, herbal medicines are the most prevalent type of treatment [3].

In developing nations, a huge number of people still rely on herbal medicine practitioners to cover their primary healthcare needs. The main arguments for using herbal medications are that they are inexpensive, conveniently available, patient-centered and closely align with the patient's beliefs [4].

The usage of herbal medicines is mainly based on experience [5]. Parallel to the recent surge in interest in herbal medicine for the prevention and treatment of various illnesses, there is growing concern regarding the safety of medicinal plants [6]. Herbal medicines are considered safe since they are natural products [7]. Some medicinal plants are safe to use up to some limit but may become toxic at higher concentrations. Some plants may show their toxic effects when mixed with other plant products. Also, there is an increasing demand for herbal formulations day by day. Raw plants are frequently treated or prescribed with other herbs in traditional medicinal practice to improve medical efficacy [8].

As a result, in the current era of evidence-based medicine, precise evaluation of the efficacy, toxicity, and appropriate usage of herbal medicines using contemporary chemical and clinical methodologies is crucial for developing traditional medicines [9]. According to AYUSH Guidelines, it is required that there should be a toxicity report for any herbal formulation to be standardized or authenticated [10]. With this in light, an effort has been made in the present paper to evaluate the safety and efficacy of some marketed herbal formulations manufactured by Shree Dhanwantri Herbals, Amritsar. Furthermore, no short or long-term toxicity study was conducted in detail to determine these formulations' lethal and safe doses. Shree Dhanwantri Herbals manufactures about 300 patent/proprietary and classical drugs, health products, beauty products, herbal extracts, *etc*.

MATERIAL AND METHODS

Collection of Material: The Herbal formulations, namely Arshonyl Capsulesule, Braintone Syrup, Fetone Syrup, F-Liv Compound Capsulesule, Rheumo Gold Capsulesule and Shvet Capsulesule, were collected from Shree Dhanwantri Herbals, Amritsar. **Table 1** lists the products' constituents and their intended usage. All herbal formulations were packaged and labeled with the scientific names of the herbal plants used in their preparation. All the formulations were checked for their expiry date before use.

TABLE 1: INGREDIENTS AND USES OF HERBAL FORMULATIONS UNDERSTUDY

Herbal formulations	Ingredients	Uses
Arshonyl Capsulesule	*Commiphora mukul, Terminalia chebula, Azadiracta indica, Allium sativum, Shorearobusta, Amorphophallus cacampanulatus, Mesua ferrea, Picrorrhiza kurroa, Sapindus trifoliatus, Zingiber officinale, Bauhinia variegate, Lauha bhasma and Heera dakhan*	Ano-rectal disorders
Braintone Syrup	*Withania somnifera, Bacopa moneiri, Convolvulus pluricaulis, Celastrus paniculatus, Glycyrrhiza galbra, Coriandrum sativum, Nardostachys jatamansi, Foeniculum vulgare, Embeliaribes, Rauwolfia sepentina, Acorus calamus*	Daily supplement for school going children and person of old age, curative in mentally retarded cases and poor intelligence, adjuvant to anti-convulsive therapy, in the condition of anxiety and depression, enuresis, asocial behavior, stress, brain fatigue syndrome, language and learning disability
Fetone Syrup	*Saraca indica, Symplocos racemosa, Withania somnifera, Allium sativum, Sida cordifolia, Ficus benghalensis, Asperagggus racemosus, Hemidesmus indicus, Salmalia malabarica, Pterocarpis santalinus, Mangifera indica, Nardostachysja tamansi, Plumbago zeylanica, Cuperusrotundus, Zingiber officinale,Triphla(Embelica officinalis, Terminalia chebula, Terminalia bellerica), Dashmoola*	Irregular menstrual cycle, dysfunctional uterine bleeding, Anovulation, Menopausal syndrome
F-Liv Compound Capsulesule	*Cyprus rotundus, Embeliaribes, Zingiber officinale, Piper longum, Piper nigrum,Arogyavardhini, PravalaPisti, Punarnavamandura, Lohabhasma, Triphlachurna, Rajatabhasma*	Anemia, general fatigue, pregnancy, liver disorders
Rheumo Gold Capsulesule	*Maharasnadin kwath, Withania somnifera, Comniphora mukul, Boswellia serrata, Moringa oliefera, Zingiber officinale, Strychnosnux-vomica, Colchicum luteum, Tinospora cordifolia, Dashmool, Vitex negundo, Ricinus*	Increases overall immunity of body, reduce pain and inflammation in joints, act as analgesic for muscles, legs, neck, shoulders, and back pain

ShvetCapsules ule	*communis Parwal Pishti, Abharakbhasma, Swarna Makshik Bhasma, Ras Sindoor, Rajat Bhasma, Swarna Bhasma Ashoka ghana, Lodhraghana, Kukkutanda twak bhasma, Sphatikabhasma, Mayaphala, Mayi, Mocharasa, Nagakesara, Puga, Kamarkas, Babbula, Kadira, Ashvatha*	Has anti-inflammatory, anti-oestrogenic, and astringent activity. Effective in management of leucorrhoea and dysfunctional uterine bleeding

Reagents and Test Kits: Serum Glutamic-Oxaloacetic Transaminase (SGOT), serum Glutamic-Pyruvic Transaminase (SGPT), and creatinine test kits were purchased from Erba Lachemas. r.o.

Experimental Animals: All the animals (weighing between 180 and 200 grams) were obtained from the National Institute of Pharmaceutical Research (NIPER), Mohali, India. The rules and regulations of control and supervision of experimental animals (CPCSEA) were followed according to the Ministry of Environment and Forests, Government of India.

***In-vivo* Acute Toxicity Studies:** Acute toxicity evaluation was conducted by following OECD-423 guidelines to find the median lethal oral dose (LD_{50}) of herbal formulations. In this study, ten (10) Wistar rats were divided into two groups (n = 5). Group 1 acted as a control and received standard feed and water *ad libitum*. The second group received a dose of test formulation (2000 mg/kg b.w.) according to the OECD guidelines, Annexure 2d(1). For syrups, the dose was decided as five times higher than the prescribed dose. Rats were observed for 24 h followed by 14 days for signs of behavioral changes and (or) mortality.

***In-vivo* Sub-chronic Toxicity Studies:** Sub-chronic toxicity evaluation was conducted following OECD-408 guidelines [11] with slight modifications. A total of twenty (20) Wistar rats were utilized for each testing formulation in this phase of the study, out of which ten (10) were male, and ten(10) were female. The rats were distributed into two groups (n = 10). Each group contains five (5) male and five (5) female rats. The first group acted as the control of normal healthy rats and was treated with vehicles only. Another group contained test animals that received a particular dose of the drug daily for ninety (90) days. Body weight was measured every week till the end of the study. The amount of prescribed human dose decided the dose. The human dose was converted into rat dose by the dose conversion formula given below.

Dose conversion formula:-

$$\text{Rat dose per kg} = \text{Human dose} / 60 \times 7.4$$

Data and Sample Collection: The rats were starved overnight before being sedated with ketamine and sacrificed at the end of the experiment. Blood was collected by retro-orbital venous plexus bleeding method [12] using capsuleillary and collected into a 5 ml blood collection tube treated with and without EDTA. The blood was then centrifuged at 5000 rpm for 10 min to obtain a clear supernatant (Serum) that was stored at -20 °C until required for biochemical analyses; conducted within a few days. The brain, lung, heart, liver, kidney, spleen, stomach, and intestine organs were collected and stored in 10% neutral buffered formalin for histopathology evaluation.

Biochemical Analyses: Serum aliquots were used to estimate the biochemical parameters. The liver function test included serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT), which were done using colorimetric methods as previously reported [13, 14]. The kidney function tests, including creatinine, were conducted according to the previously reported methods [15] and with the aid of commercially available test kits (Erba Lachemas.r.o.). Blood parameters were done within three hours of the blood collection by using an automatic hematology analyzer (Medonic M-series). The parameters evaluated were total leucocyte count, total erythrocyte count, hemoglobin, platelet count, neutrophils, lymphocytes, eosinophils, and monocytes.

Histopathological Evaluation: The fixed, collected organs in formal saline were chopped into 5 mm thick slices and dehydrated with graded concentrations of ethanol (70, 95, and 99%: absolute ethanol); cleared in xylene, and embedded in paraffin wax.

The embedded tissues (Brain, Lung, Heart, Liver, Kidney, Spleen, Stomach, and Intestine) were sectioned at 6 *μm* thickness, stained with hematoxylin and eosin (H & E) and examined under the light microscope according to the methods described [16]. The sections were photographed at a magnification of 100x with the microscope (Nikon ECLIPSE T*i*2).

Statistical Analyses: The mean and standard deviation (Mean±SD) are used to express the data received from the investigation. Statistical differences between means of control groups and test groups were evaluated by paired *t*-test and one-way analysis of variance (ANOVA). Dunnett's multiple comparison test was used to compare different groups. Differences in means were considered significant at $P<0.05$.GraphPad Prism®, version 5 was used for all statistical analyses.

RESULTS:

Acute Toxicity Study: All the animals were dosed at 2000 mg/kg survived till the end of the study (14 days). As per the OECD-423 guidelines, the LD_{50} was 5000 mg/kg or morefor each formulation (Supplementary **Fig. 1**).

Effect on body Weight, Behavioral Changes and Mortality: All animals under study were observed for changes in body weight, mortality, and different behavioral patterns such as respiratory patterns, lethargy, tremors, and gait. No significant change in body weight of animals was observed **Fig. 1**. In case of behavioral observations, all animals have shown normal behavior. Also, no any mortality occurred throughout the study.

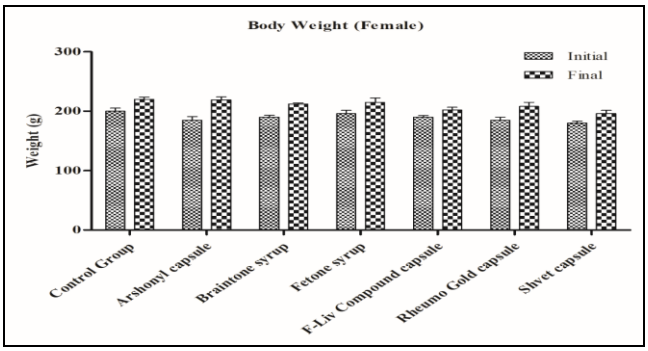

FIG. 1: EFFECT OF HERBAL FORMULATIONS ON BODY WEIGHT IN FEMALE WISTAR RATS UNDER ACUTE TOXICITY STUDY. DATA SHOWN HERE INCLUDES MEAN±SD.

Sub Chronic Toxicity:

Effect on Body Weight and Behavioral Changes: All animals under study were observed for different behavioral patterns such as respiratory pattern, lethargy, tremors, gait and mortality. Data presented in **Table 3** shows the behavioral observations of animals under the sub-chronic toxicity study.

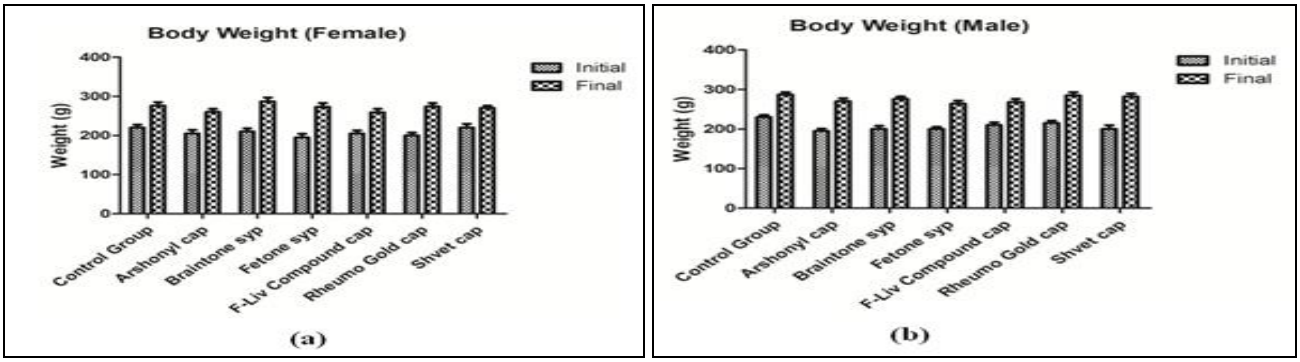

FIG. 2: EFFECT OF HERBAL FORMULATIONS ON BODY WEIGHT IN MALE (A), AND FEMALE (B) WISTAR RATS. Data shown here includes Mean±SD.

Effect on Liver and Kidney Function Parameters: In the case of male rats, there was no significant difference ($p<0.05$, $p<0.01$, $p<0.001$) observed in serum creatinine level in test animals of Arshonylcapsule, Fetonesyrup, F-Liv Compound capsule, Rheumo Gold capsule and Shvetcapsule. In test animals of Braintone syrup, it was significantly higher ($p<0.05$) compared with the control group. Also in the case of SGOT, no significant difference ($p < 0.05$) was obtained when test animals of all herbal formulations were compared with that of the control group. There was a significant increase ($p<0.001$) in serum SGPT levels of test animals of Arshonylcapsule, Braintonesyrup, Fetonesyrup and Rheumo Gold capsule as compared to the control group and in the

case of F-Liv Compound capsule and Shvetcapsule, no significant difference ($p<0.05$, $p<0.01$, $p<0.001$) was obtained. In female rats, serum creatinine level was not significantly different ($p<0.05$, $p<0.01$, $p<0.001$) from the control group. There was no significant difference ($p<0.05$, $p<0.01$, $p<0.001$) in serum SGOT levels observed. Serum SGPT levels were significantly higher ($p<0.001$) in test animals of Arshonylcapsule, Braintonesyrup, Fetonesyrup, and Shvetcapsule. While in the case of test animals of Rheumo Gold capsule, serum SGPT level was significantly less ($p<0.001$) than that of the control group **Fig. 3** and **Fig. 4.**

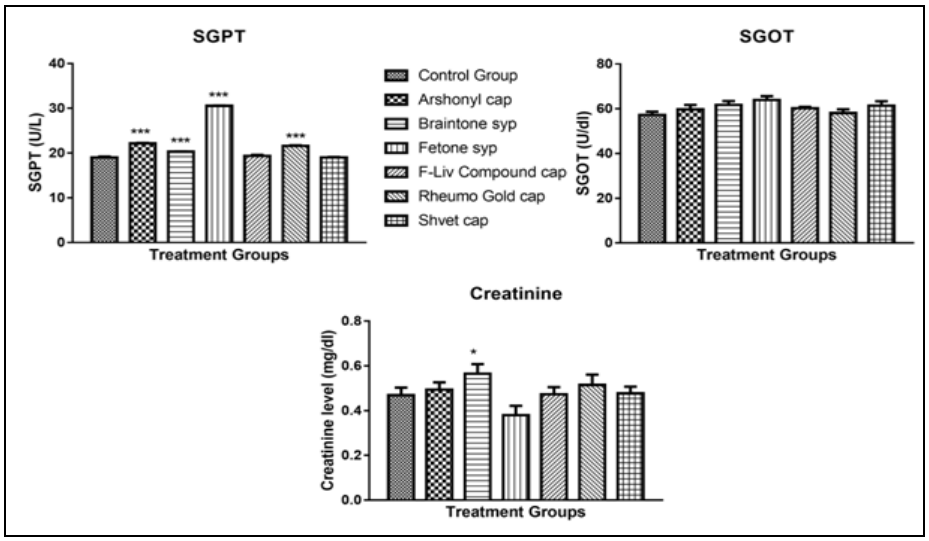

FIG. 3: EFFECT OF HERBAL FORMULATIONS ON THE LIVER AND KIDNEY FUNCTION PARAMETERS IN MALE WISTAR RATS. Values are in Mean±SD. Values shown with an asterisk represent significant differences compared with the control group. *p<0.05, **p<0.01, ***p<0.001.

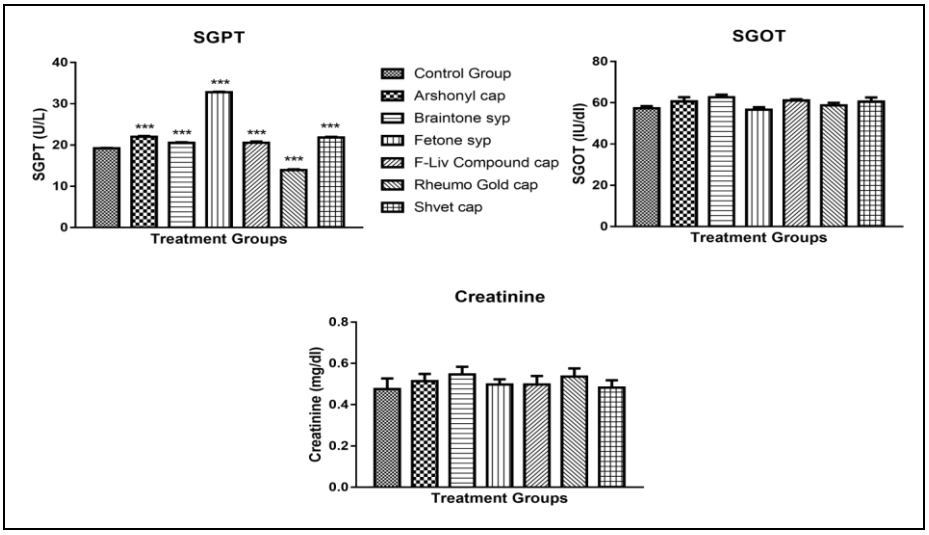

FIG. 4: EFFECT OF HERBAL FORMULATIONS ON THE LIVER AND KIDNEY FUNCTION PARAMETERS IN FEMALE WISTAR RATS. Values are in mean±SD. Values shown with an asterisk represent significant differences compared with the control group. *p<0.05, **p<0.01, ***p<0.001.

Effect on Blood Parameters: In the case of male rats, the Hb content of test animals of Arshonylcapsule and Fetonesyrup was not significantly different ($p<0.05$, $p<0.01$, $p<0.001$) from that of the control group. However, the Hb content of test animals of Braintonesyrup ($p<0.01$), F-Liv Compound capsule ($p<0.001$), Rheumo Gold capsule ($p<0.01$) and Shvetcapsule ($p<0.01$) was significantly less than the control group. There was no significant difference ($p<0.05$, $p<0.01$, $p<0.001$) in RBC count in test animals of all herbal formulations except the F-Liv Compound capsule ($p<0.01$). No significant difference ($p<0.05$, $p<0.01$, $p<0.001$) was observed in the WBC Count

of all tested herbal formulations except the F-Liv Compound capsule ($p<0.05$). There was no significant difference in Platelet Count, Eosinophil Count, Neutrophil and Lymphocyte Count of test animals of herbal formulations from the control group. Monocyte count was significantly less in test animals of Rheumo Gold capsule ($p<0.05$) from the control group while in test animals of other herbal formulations, it was not significantly different ($p<0.05$, $p<0.01$, $p<0.001$) as compared to control group **Table 4A** And **Table 4B.** In the case of female rats, all blood parameters [Hb, RBC, WBC, Platelet count, Neutrophil count, Lymphocyte count, Monocyte count and Eosinophil count] of test animals of all formulations under study were not significantly different ($p<0.05$, $p<0.01$, $p<0.001$) from the control group.

TABLE 2A: RESULTS OF HEMATOLOGICAL PARAMETERS OBSERVED IN MALE WISTAR RATS FOR SUB-CHRONIC TOXICITY STUDY

Hematological parameter	Control group (Mean±SD)	Arshonyl capsule (Mean±SD)	Braintone syrup (Mean±SD)	Fetone syrup (Mean±SD)	F-Liv Compound capsule (Mean±SD)	Rheumo Gold capsule (Mean±SD)	Shvetcapsule (Mean±SD)
Hb content (g/dl)	15.62±0.10	14.96±0.37	14.73±0.45 **	15.22±0.10	14.82±0.26 ***	14.92±0.55 **	14.96± 0.18 **
RBC Count (10^6/mm^3)	8.3±0.43	8.3±0.40	7.59±0.11	8.6±0.43	6.78±0.26 **	8.2±0.3	7.97±0.83
WBC Count (10^3/mm^3)	12.4±0.45	11.35±0.45	13.20±0.721	11.27±0.65	10.59±1.10 *	11.66±0.51	10.90±0.70
Platelet Count (10^3/mm^3)	300±19.2	340±18	340±22.3	343±26.3	332.66±17	344±22.06	305±25.2
Neutrophil Count (10^3/mm^3)	2.69±0.31	2.74±0.21	2.71±0.09	2.35±0.15	2.29±0.31	2.54±0.58	2.44±0.12
Lymphocyte Count (10^3/mm^3)	6.77±0.24	6.42±0.14	6.74±0.77	6.79±0.42	6.05±0.09	6.30±0.30	6.09±0.09
Monocyte Count (10^3/mm^3)	0.017± 0.00030	0.016± 0.00056	0.019± 0.0016	0.018± 0.0007	0.017± 0.0017	0.015± 0.0002 *	0.019±0.0013
Eosinophil Count (10^3/mm^3)	0.038±0.006	0.034±0.005	0.04±0.0061	0.0395± 0.0061	0.038± 0.002	0.032± 0.004	0.04±0.005

Values are in Mean±Sd. values shown with an asterisk represent significant differences compared with the control group. *p<0.05, **p<0.01, ***p<0.001.

TABLE 2B: RESULTS OF HEMATOLOGICAL PARAMETERS OBSERVED IN FEMALE WISTAR RATS FOR SUB-CHRONIC TOXICITY STUDY

Hematological parameter	Control group (Mean±SD)	Arshonyl capsule (Mean±SD)	Braintone syrup (Mean±SD)	Fetone syrup (Mean±SD)	F-Liv Compound capsule (Mean±SD)	Rheumo Gold capsule (Mean±SD)	Shvetcapsule (Mean±SD)
Hb content (g/dl)	15±0.25	14.73±0.20	14.13±0.55	15±0.251	14.06±0.77	14.96±0.28	14.07±0.20
RBC Count (10^6/mm^3)	7.5±0.36	7.46±0.40	6.64±0.045	7.5±0.36	6.76±0.832	7.4±0.26	7.3±0.20
WBC Count (10^3/mm^3)	11.3±0.52	11.1±0.45	11.82±0.81	11.37±0.56	10.17±0.76	10.90±0.30	10.85±0.55
Platelet Count (10^3/mm^3)	335±19	368±22	366.5±23.6	328±24.3	324.5±24.44	351±10.0	326.75±24
Neutrophil Count (10^3/mm^3)	2.09±0.20	2.23±0.09	2.37±0.26	2.42±0.28	2.01±0.04	2.03±0.08	2.03±0.11
Lymphocyte Count (10^3/mm^3)	6.42±0.25	6.23±0.14	5.93±0.86	6.68±0.36	5.8±0.18	6.03±0.65	6.61±0.14
Monocyte Count (10^3/mm^3)	0.015±0.004	0.015±0.003	0.018±0.002	0.019±0.002	0.016±0.004	0.014±0.001	0.017±.0.001
Eosinophil Count (10^3/mm^3)	0.05±0.006	0.043±0.009	0.055±0.009	0.055±0.0092	0.050±0.003	0.043±0.004	0.059±0.001

Values are in Mean±SD. Values shown with an asterisk represent significant differences compared with the control group. *p<0.05, **p<0.01, ***p<0.001.

Results of Histopathological Data: Histo-pathological studies of all organs (Brain, Lung, Heart, Liver, Kidney, Spleen, Stomach and Intestine) have shown that no sign of toxicity was found in any of the organs. Histology of organs was normal **Fig. 5-11.**

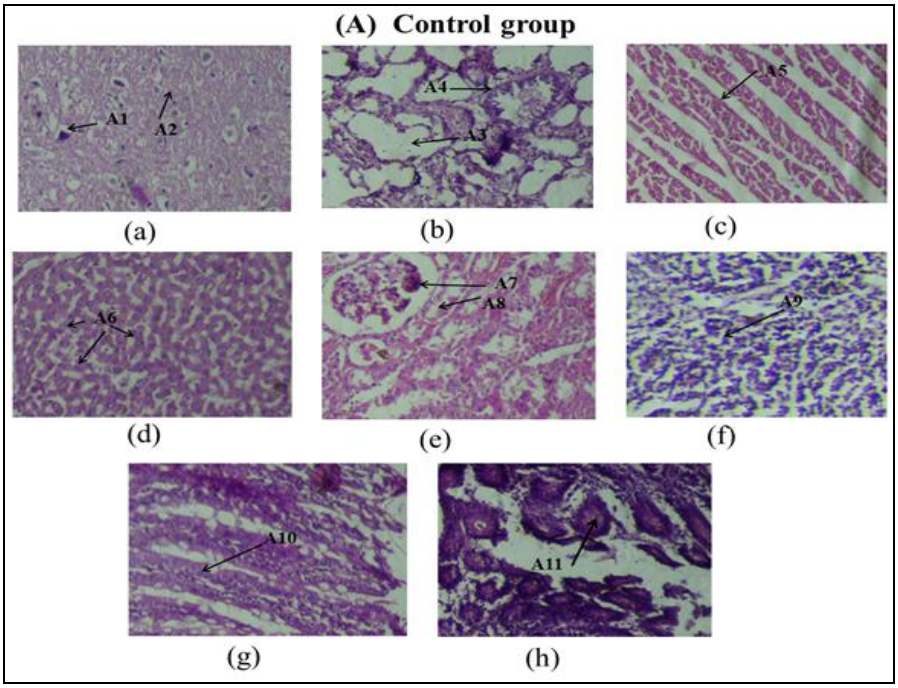

FIG. 5: HISTOLOGICAL DETAILS OF (A) CONTROL GROUP (A) BRAIN (A1-NORMAL GLIAL CELLS, A2-FIBRILLARY MATERIAL OF BRAIN WITH NORMAL ARCHITECTURE), (B) LUNG (A3- ALVEOLAR SAC AND A4-ALVEOLAR LINING), (C) HEART (A5- NORMAL CARDIAC MUSCLE), (D) LIVER (A6- NORMAL HEPATOCYTES), (E) KIDNEY (A7- GLOMERULUS AND A8- COLLECTING TUBULES, (F) SPLEEN (A9-LYMPHOID STRUCTURES), (G) STOMACH (A10- FOVELOAR EPITHELIUM OF STOMACH) AND (H) INTESTINE (A11- MUCOSAL GLANDS OF THE INTESTINE)

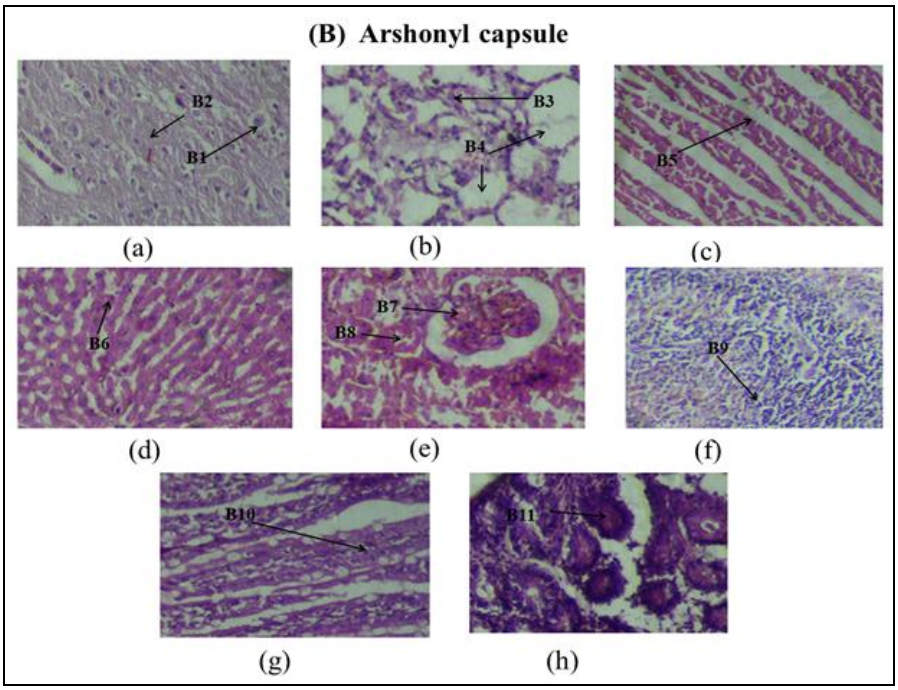

FIG. 6: HISTOLOGICAL DETAILS OF (B) ARSHONYL CAPSULE (A) BRAIN (B1- GLIAL CELLS AND B2-FIBRILLARY MATERIAL), (B) LUNG (B3- ALVEOLAR LINING AND B4-ALVEOLUS SAC), (C) HEART (B5-CARDIAC MUSCLE), (D) LIVER (B6- NORMAL HEPATOCYTE), (E) KIDNEY (B7-NORMAL GLOMERULUS AND B8- COLLECTING TUBULE) AND (F) SPLEEN(B9-LYMPHOID STRUCTURES OF THE SPLEEN), (G) STOMACH (B10- FOVEOLAR EPITHELIUM) AND (H) INTESTINE (B11- MUCOSAL GLANDS)

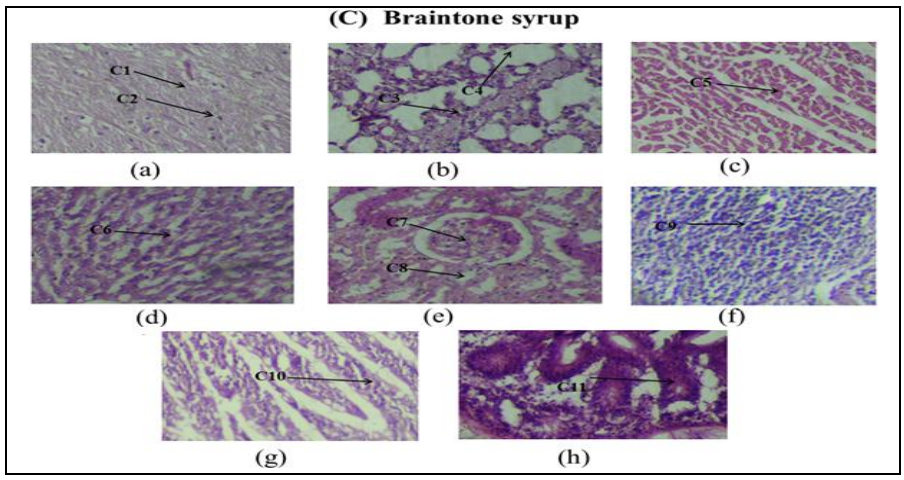

FIG. 7: HISTOLOGICAL DETAILS OF (C) BRAINTONE SYRUP (A) BRAIN (C1- GLIAL CELLS AND C2-FIBRILLARY MATERIAL OF BRAIN), (B) LUNG (C3-ALVEOLAR LINING AND C4-ALVEOLUS), (C) HEART (C5-CARDIAC MUSCLE), (D) LIVER (C6- HEPATOCYTES), (E) KIDNEY (C7- GLOMERULUS AND C8- TUBULES), (F) SPLEEN (C9- LYMPHOID STRUCTURES OF SPLEEN), (G) STOMACH (C10- FOVEOLAR EPITHELIUM) AND (H) INTESTINE (C11- MUCOSAL GLANDS)

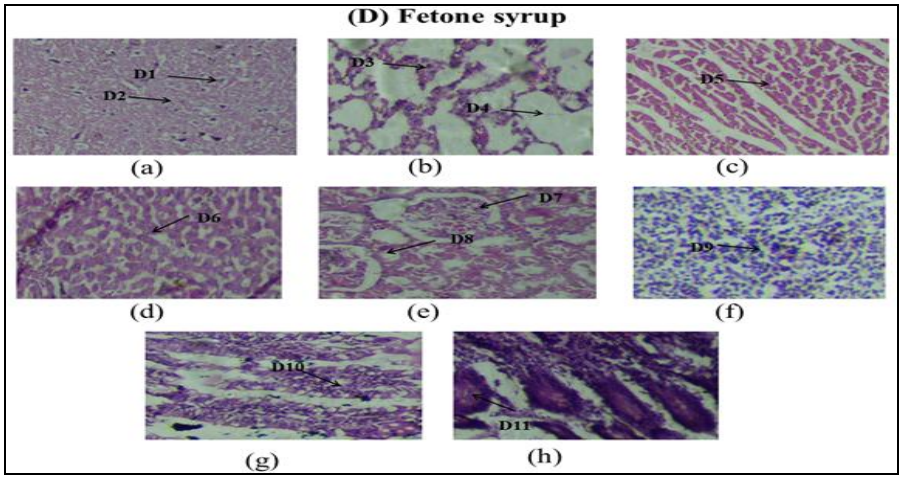

FIG. 8: HISTOLOGICAL DETAILS OF(D) FETONE SYRUP (A) BRAIN (D1-GLIAL CELL AND D2-FIBRILLARY MATERIAL), (B) LUNG (D3- ALVEOLAR LINING AND D4-ALVEOLUS), (C) HEART (D5-CARDIAC MUSCLE), (D) LIVER (D6- HEPATOCYTES), (E) KIDNEY (D7- GLOMERULUS AND D8- TUBULES), (F) SPLEEN (D9- THE NORMAL LYMPHOID STRUCTURE OF SPLEEN), (G) STOMACH (D10- NORMAL FOVEOLAR EPITHELIUM) AND INTESTINE (D11-NORMAL MUCOSAL GLANDS)

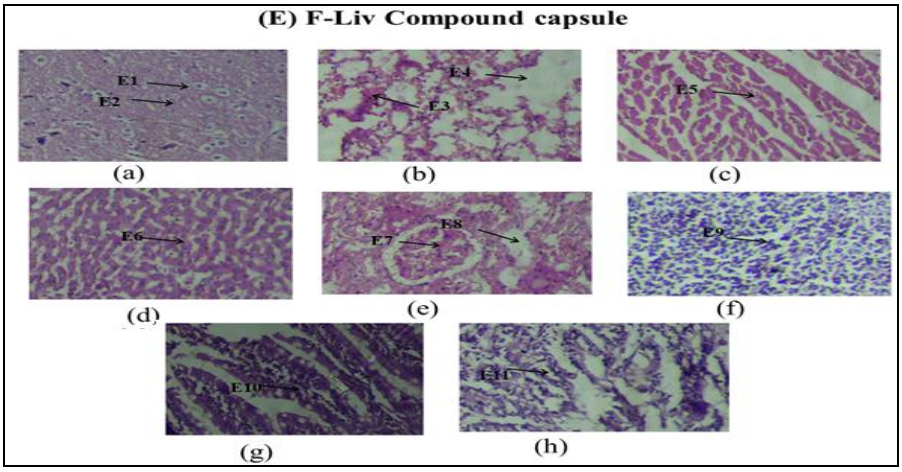

FIG. 9: HISTOLOGICAL DETAILS OF (E) F-LIV COMPOUND CAPSULE (A) BRAIN (E1-GLIAL CELL AND E2-FIBRILLAR MATERIAL), (B) LUNG (E3-ALVEOLAR LINING AND E4- ALVEOLAR SAC), (C) HEART (E5-CARDIAC MUSCLE), (D) LIVER (E6-HEPATOCYTE), (E) KIDNEY (E7- GLOMERULUS AND E8- TUBULES), (F) SPLEEN(E9- LYMPHOID STRUCTURES OF THE SPLEEN), (G) LIVER (E10- FOVEOLAR EPITHELIUM AND (H) INTESTINE(E11- MUCOSAL GLANDS)

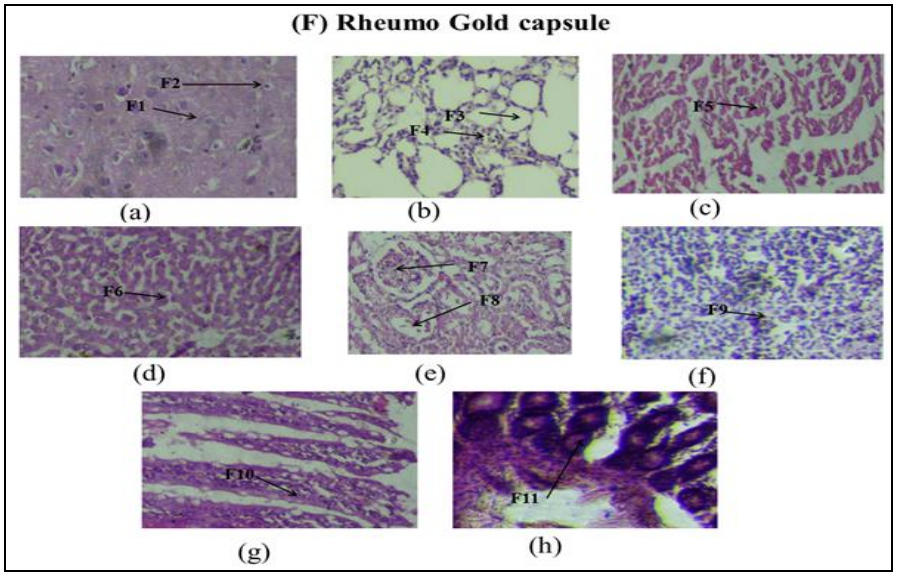

FIG. 10: HISTOLOGICAL DETAILS OF(F) RHEUMO GOLD CAPSULE (A) BRAIN (F1- FIBRILLARY MATERIAL AND F2- GLIAL CELLS), (B) LUNG (F3-ALVEOLAR SAC AND F4- ALVEOLAR LINING), (C) HEART (F5- CARDIAC MUSCLE), (D) LIVER (F6- NORMAL HEPATOCYTES), (E) KIDNEY (F7-GLOMERULUS AND F8- TUBULES), (F) SPLEEN (F9- LYMPHOID STRUCTURES OF SPLEEN), (G) STOMACH (F10- FOVEOLAR EPITHELIUM) AND INTESTINE(F11- NORMAL MUCOSAL GLANDS)

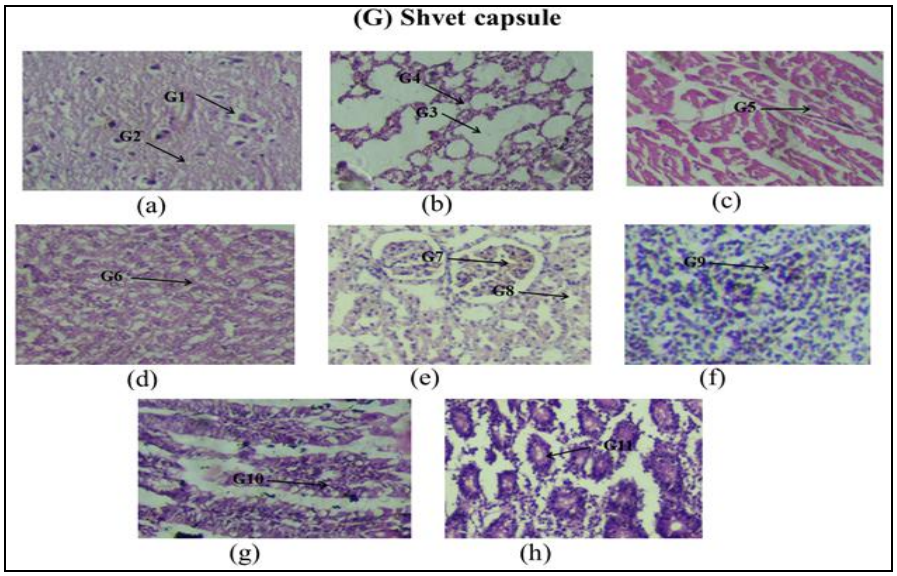

FIG. 11: HISTOLOGICAL DETAILS OF(G) SHVETCAPSULE (A) BRAIN (G1-GLIAL CELL AND G2-FIBRILLARY MATERIAL), (B) LUNG (G3-ALVEOLAR SAC AND G4- ALVEOLAR LINING), (C) HEART (G5-CARDIAC MUSCLE), (D) LIVER (G6- HEPATOCYTES), KIDNEY (G7-GLOMERULUS AND G8- TUBULES), SPLEEN (G9-LYMPHOID STRUCTURES OF THE SPLEEN), STOMACH (G10-FOVEOLAR EPITHELIUM) AND INTESTINE (G11-MUCOSAL GLANDS OF THE INTESTINE)

DISCUSSION: Herbal formulations are nowadays gaining more attention due to their long-lasting biological effects. It was also considered that herbal formulations have fewer side effects. But AYUSH ministry has made it mandatory to evaluate the safety profile of herbal formulations keeping in mind the few cases where herbal formulations were reported to have toxicity [17]. Toxicity studies are essential criteria for pharmacological evaluation of herbal formulations. Acute oral toxicity of all herbal formulations showed no mortality even at its highest dose levels (2000 mg/kg). A toxicity study helps evaluate the substance's nature, whether it's toxic or non-toxic[18]. Acute toxicity studies in rodents offer data on the therapeutic index and provide the absolute dose for pharmacological assays [19]. The OECD rules for chemical labeling and classification of acute toxicity based on oral LD_{50} values revealed that LD_{50} values for all herbal formulations were greater than 5000 mg/kg body

weight, placing them in category 5. If body weight is examined regularly and properly during the study, it can be one of the most sensitive markers of an animal's condition. During toxicity studies, mortality and abnormal behavioral changes were not observed. According to our findings, the herbal formulations had no acute oral toxicity in rats.

The effect of the test material on animals is evaluated for 90 days in a sub-chronic toxicity study. It aids in evaluating test drugs that are administered for a longer period [20]. All of the herbal formulations were given to the animals for 90 days, and they were evaluated for behavioral changes and body weight changes throughout the trial. At the end of the study, all animals were sacrificed, biochemical analysis was done, and histopathology of organs was studied. In the repeated dose sub-chronic oral toxicity study, the herbal formulations did not induce any signs of morbidity or mortality. Any changes in body weight can be used as a rapid assessment for the side effects of a drug [21].

There was a significant change ($P < 0.05$) in animals' initial and final body weights. These results confirmed that NCEF did not affect the growth retardation and appetite of rats. An increase in weight does not indicate any kind of toxicity; animals naturally mature with and gain weight; instead decrease in weight can be a deleterious sign [22]. Biochemical analysis was also done to reveal the biochemical changes caused by herbal formulations if any. In this study, there were no significant changes in ($P < 0.05$) Creatinine levels in female rats were observed, whereas in the case of male rats, there were significant changes in test animals of Braintone syrup at the level of $P<0.001$. Serum SGOT levels in both male and female rats were not significantly different in test animals of formulations ($p<0.05$, $p<0.01$, $p<0.001$) from the control group. This implies that no toxic effects were produced by herbal formulations on the kidney and liver in most of the tested herbal formulations. In female rats, serum SGPT levels were significantly different ($p<0.001$) in test animals of all herbal formulations as compared to control. In male rats, serum SGPT level was not significantly different in test animals of F-Liv Compound capsule and Shvetcapsule from control group. In contrast, SGPT level was significantly different ($p<0.001$) in Arshonyl capsule, Braintonesyrup, Fetonesyrup, Rheumo Gold capsule. However, significant biochemical alterations in SGPT level were observed at the level of ($p<0.001$), and histopathological data did not show any sign of toxicity in the liver as the architecture of hepatocytes was normal.

The blood parameters (Hb, RBC, WBC, Platelets, Neutrophils, Lymphocytes, Eosinophil, and Monocytes) were also evaluated. A significant decrease was observed in Hb content of male animals treated with Braintone syrup, F-Liv Compound capsule, Rheumo Gold capsule, and Shvet. Similarly, in the case of WBC ($P<0.01$), RBC ($P<0.05$), and Monocyte count ($P<0.05$) significant changes were observed. As the decrease in RBC count is also observed in case of over hydration and is a normal condition, an increase in RBC than normal can be dangerous and lead to various problems such as heart conditions, bone marrow disease, and kidney diseases [23].

A mild decrease in Hb, WBC, and Monocyte count is not directly linked with toxicity. It can be due to other reasons such as lower immunity. These mild alterations cannot be considered signs of toxicity as histopathology of all organs showed no necrosis and infiltrations of lymphocytes in any of the tissue.

It showed that herbal formulations did not have any significant difference in urine output at an administered dose in test animals of all herbal formulations as compared to control. It showed that these herbal formulations did not produce a diuretic effect which is a good indication.

Histopathological data of organs (Brain, Lung, Kidney, Stomach, Heart, Intestine, Stomach, and Spleen) showed no signs of toxicity. Although no mortality was observed in the study, it is suggested that recommended dose of these herbal formulations should be reduced as it resulted in significant changes in serum SGPT level and some of the blood parameters. All of this data points toward the potential safety of these herbal formulations.

CONCLUSION: As per the results obtained from the present study, there was no mortality occurred the dose 2000 mg/kg b.w. Therefore LD_{50} of all the

herbal formulations was found to be more than 5000 mg/kg b.w. These formulations did not show any abnormal behavioral pattern in acute and subchronic activity during the study. Creatinine and SGOT levels were not significantly different. However, SGPT level was found to be altered in sub-chronic activity in both male and female animals. The architecture of liver cells was unaltered and did not show any signs of toxicity. Histopathology of other organs was also normal. So, these herbal formulations are safe as these were not observed to have any kind of toxicity.

Supplementary Files:

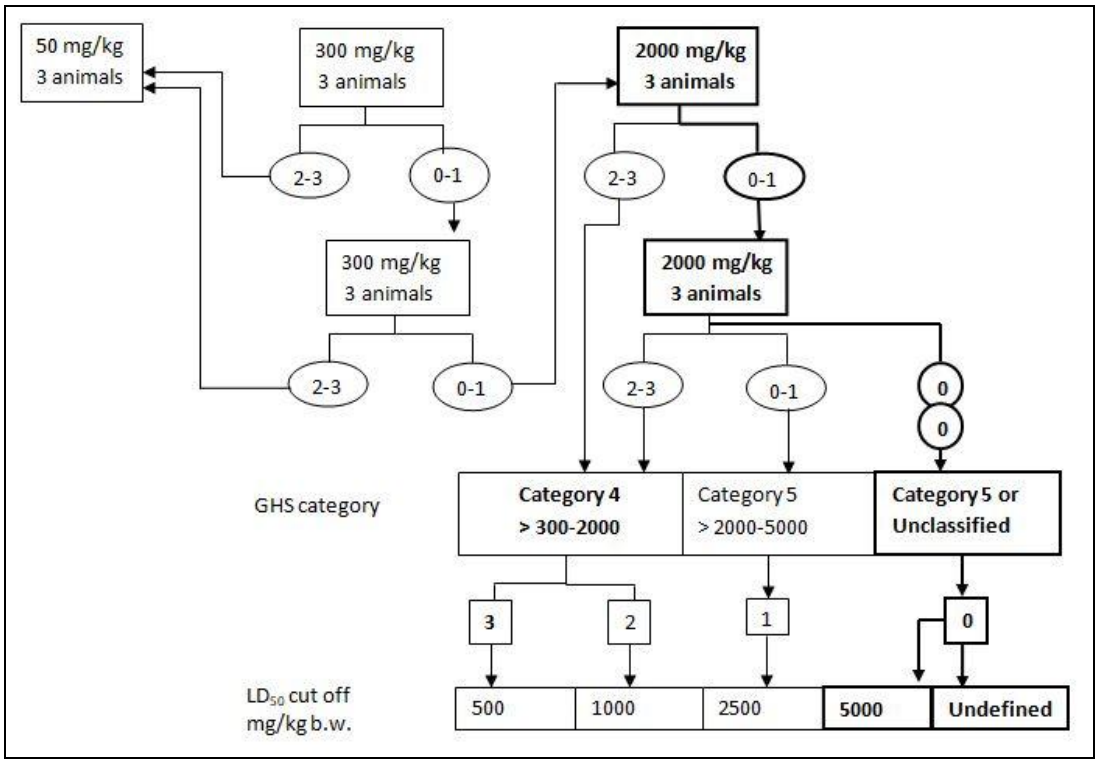

FIG. S1: CALCULATIONS OF LD_{50} VALUES ACCORDING TO THE OECD-423 GUIDELINES. AT A DOSE 2000 MG/KG B.W., ALL THE ANIMALS SURVIVED TILL THE END OF 14 DAYS OF STUDY. ACCORDING TO THE SCHEME PROVIDED BY GUIDELINES TO REDUCE THE NUMBER OF ANIMALS USED FOR THE STUDY, LD_{50} WAS CALCULATED AS 5000 mg/kg OR MORE

Author Contribution: PS, AR and SA conceived the idea; GS provided the material; PS and AR performed the experimental work and wrote the paper; BS and SA reviewed the manuscript and made the corrections. All the authors approved the final manuscript.

Funding: This study was funded by Shree Dhanwantri herbals, Amritsar.

ACKNOWLEDGMENTS: We acknowledge Gurmeet Singh and Management from Shree Dhanwantri Herbals for financial and other technical support, Manisha Sharma from the Department of Pathology, Sri Guru Ram Das Institute of Medical Science and Research, Amritsar for evaluation of slides of histopathology, Hasandeep Singh (Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar) and Davinder Singh (Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar) for guiding the work and Guru Nanak Dev University for providing research infrastructure.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

REFERENCES:

1. Karole S, Shrivastava S, Thomas S, Soni B, Khan S, Dubey J, Dubey SP, Khan N and Jain DK: Polyherbal formulation concept for synergic action: a review. Journal of Drug Delivery and Therapeutics 2019; 9(1): 453-66.
2. Khan MS and Ahmad I: Herbal medicine: current trends and future prospects. New Look to Phytomedicine 2019; 3-13.

3. Ang L, Lee HW, Choi JY, Zhang J & Lee MS: Herbal medicine and pattern identification for treating COVID-19: a rapid review of guidelines. Integrative Medicine Research 2020; 9(2): 100407.
4. Kamel FO, Magadmi RM, Hagras MM, Magadmi B and AlAhmad RA: Knowledge, attitude and beliefs toward traditional herbal medicine use among diabetics in Jeddah Saudi Arabia. Complementary Therapies in Clinical Practice 2017; 29: 207-12.
5. Nguyen PH, De Tran V, Pham DT, Dao TN and Dewey RS: Use of and attitudes towards herbal medicine during the COVID-19 pandemic: a cross-sectional study in Vietnam. European Journal of Integrative Medicine 2021; 44: 101328.
6. Bernstein N, Akram M, Yaniv-Bachrach Z and Daniyal M: Is it safe to consume traditional medicinal plants during pregnancy. Phytotherapy Research 2021; 35(4): 1908-24.
7. Elachouri M, Kharchoufa L, Fakchich J, Lorigooini Z, Subhasis P and Subhash M: Ancestral phytotherapeutic practices in Morocco: regards on history, current state, regulatory and safety of commonly used herbal medicine. Arabian Journal of Chemical and Environmental Research. 2021; 8(1): 133-49.
8. Zhang J, Hu K, Di L, Wang P, Liu Z, Zhang J, Yue P, Song W, Zhang J, Chen T and Wang Z: Traditional herbal medicine and nanomedicine: Converging disciplines to improve therapeutic efficacy and human health. Advanced Drug Delivery Reviews 2021; 178: 113964.
9. Shan QY, Sang XN, Hui H, Shou QY, Fu HY, Hao M, Liu KH, Zhang QY, Cao G and Qin LP: Processing and polyherbal formulation of *Tetradium ruticarpum* (A. Juss.) Hartley: Phytochemistry Pharmacokinetics and Toxicity Frontiers in Pharmacology 2020; 11: 133.
10. General guidelines for safety/toxicity evaluation of Ayurvedic formulations. First edition 2018. Accessed May 20, 2022. https://www.ayush.gov.in/docs/guideline_safety_toxicity.pdf
11. OECD guidelines 408. Revision 2018. Accessed May 20, 2022. https://www.oecd.org/env/ehs/testing/Revision%20TG%20408%202018.pdf
12. Kumari M, Bajad SM, Kshirsagar SR, Chinde S, Balaji AS, Kumar MJ, Saxena S and Kumari SI: Sub-chronic oral toxicity evaluation of herbo-metallic formulation Arshakuthar rasa in rats. Journal of Ethnopharmacology 2022; 115306.
13. Reitmen S: A colorimetric method for the determination of serum glutamate oxaloacetate and serum glutamate pyruvate transminase. Am J Clin Pathol 1957; 28:56.
14. Pruden EL: Clinical guide to laboratory tests. Tietz NW, Finley PR, editors. Philadelphia: WB Saunders Company 1995; 4.
15. Bowers LD and Wong ET: Kinetic serum creatinine assays. II. A critical evaluation and review. Clinical Chemistry 1980; 26(5): 555-61.
16. Gurr E: Methods of analytical histology and histo-chemistry. Leonard Hill 1958.
17. Mensah ML, Komlaga G, Forkuo AD, Firempong C, Anning AK and Dickson RA: Toxicity and safety implications of herbal medicines used in Africa. Herbal Medicine 2019; 63: 1992-0849.
18. Krewski D, Andersen ME, Tyshenko MG, Krishnan K, Hartung T, Boekelheide K, Wambaugh JF, Jones D, Whelan M, Thomas R and Yauk C: Toxicity testing in the 21st century: progress in the past decade and future perspectives. Archives of Toxicology 2020; 94(1): 1-58.
19. Gadaleta D, Vuković K, Toma C, Lavado GJ, Karmaus AL, Mansouri K, Kleinstreuer NC, Benfenati E and Roncaglioni A: SAR and QSAR modeling of a large collection of LD50 rat acute oral toxicity data. Journal of Cheminformatics 2019; 11(1): 1-6.
20. Wang Y, Wang H, Zhang L, Zhang Y, Sheng Y, Deng G, Li S, Cao N, Guan H, Cheng X and Wang C: Subchronic toxicity and concomitant toxicokinetics of long-term oral administration of total alkaloid extracts from seeds of *Peganum harmala* Linn: A 28-day study in rats. Journal of Ethnopharmacology 2019; 238: 111866.
21. Kpemissi M, Metowogo K, Melila M, Veerapur VP, Negru M, Taulescu M, Potârniche AV, Suhas DS, Puneeth TA, Vijayakumar S and Eklu-Gadegbeku K: Acute and subchronic oral toxicity assessments of *Combretum micranthum* (Combretaceae) in Wistar rats. Toxicology Reports 2020; 7: 162-8.
22. Unuofin JO, Otunola GA and Afolayan AJ: Evaluation of acute and subacute toxicity of whole-plant aqueous extract of *Vernonia mespilifolia* Less. in Wistar rats. Journal of Integrative Medicine 2018; 16(5): 335-41.
23. Ebohon O, Irabor F and Omoregie ES: Sub-acute toxicity study of methanol extract of *Tetrorchidiumdidymo stemon* leaves using biochemical analyses and gene expression in Wistar rats. Heliyon 2020; 6(6): 04313

How to cite this article:
Singh B, Sharma P, Rajput A, Singh G and Arora S: Evaluation of acute and subchronic toxicity of polyherbal formulations of Shree Dhanwantri herbals in Wistar rats. Int J Pharm Sci & Res 2022; 13(12): 5068-79. doi: 10.13040/IJPSR.0975-8232.13(12).5068-79.