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Evaluation of Acute Toxicity and Hepatoprotective Activity of Scrofoloso - 5 (S5) and Livome, Electro Homoeopathic Herbal Preparations against CCl_4 Induced Liver Toxicity

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PSB designed the study, performed biochemical study and the statistical analysis, and wrote the first draft of the manuscript. Author VK wrote the protocol. Authors AS and RB managed the analyses of the histopathological study and the author Venkatesh involved in animal studies and author KP carried out review of literature. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Many medical professionals are using electro homoeopathic preparations which are available in Indian market. In the present study, the electro homoeopathic medicines Scrofoloso 5 (S5) and

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Livome were evaluated for their acute toxicity and hepatoprotective activity against CCl_4 induced toxicity.

Study Design: For acute toxicity studies six groups with six mice each one was used to evaluate two test samples. Group I,II and III were administered test sample S5 in the dose 0.25ml,0.5ml and 1.0ml respectively and Group IV,V and VI were administered test sample Livome with the dose 0.25ml,0.5ml and 1.0ml respectively. Behaviors were studied up to 14 days. For hepatoprotective evaluation 5 groups of six rats Group I, II III, IV and V were employed and biochemical and histological profile were studied.

Materials and Methods: The electro homoeopathic preparations of Scrofoloso-5 (S5) and Livome were purchased from the local market. The acute toxicity was evaluated in female swiss albino mice, while the hepatoprotective efficacy was screened in male Wistar rats. The liver function biochemical parameters and the histopathological profile were used as criteria for hepatoprotective estimation.

Results: There was no acute toxicity for both S5 and Livome drugs up to high dose of 1.0 ml/day for mice of 25g b.wt.orally. The hepatoprotective activity of S5 was similar to the standard hepatoprotective drug silymarin and Livome effects are more significant than the standard hepatoprotective drug silymarin. The histological profile of the liver tissue showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration as similar to the controls.

Conclusion*:* The Electro homoeopathic medicines available in market for the Electro homoeopathic practitioners S5 and Livome afforded significant protection from CCl_4 induced liver damage.

Keywords: Electro homoeopathy; acute toxicity; hepatoprotective; scrofoloso 5; livome.

1. INTRODUCTION

The medicinal plants have played a significant role in the most convenient and effective health care. Many of the currently available drugs were derived directly or indirectly from phytochemicals. The human body requires essential chemicals and minerals for metabolism and all these are obtained from plants they keep the blood and the lymph in the purified state [1]. All plants receive and store energy from sun-light, rain and earth in lesser or greater quantity in the form of chemicals, minerals and charged ionic electrolytes which can be taken out from the plants in the form of extracts and spagyric essences and used to correct the metabolic activities. These plant extracts and spagyric essences are known as medicinal herbal drugs. There are various medical systems developed in the different geographic regions of the world based on experience and observation on traditional usage of herbal medicine and medication over the time such as Ayurveda, Siddha, Unani, Chinees, Homoeopathy, electro homoeopathy and other traditional of folklore medical systems. In 19th century Italian Count Ceaser Mattei (1809-1896), borrowed from Paracelsus the process of preparing the vegetable substances by means of a more or less complicated mode of separation, purification and cohobation, and also the final combination of a number of ingredients with similar or supplementary effect to form a compound medicinal unity and proposed the ideology that compound medicines required for the complexity of the symptoms [2]. This ideology may be more acceptable in the current scenario due to advancement in techniques and tools and their applications in biomedical research at genomic and proteomic level showed that multiple gene regulation involved in metabolic pathway for pathological condition or to restore the normal biochemical and physiological condition of the body from the pathological conditions.

Our survey revealed that presently hundreds of Electro homoeopathic institutions and lacks of practitioners and millions of beneficiaries exist in all over the world including India. There are number of electro homoeopathic medicines available in the market in India for public. However, Scrofoloso 5 and Livome electro homoeopathic medicinal preparations are widely using for the treatment of jaundice by electro homoeapathic practioners in Karnataka and other states in India. However, medicinal value of publicly available electro homoeopathic Scrofoloso 5 and Livome products pertaining to acute toxicity and hepatoprotective activity has not yet reported, and is the focus of our study.

2. MATERIALS AND METHODS

2.1 Samples, Chemicals and Animals

2.1.1 Electro homoeopathic medicines collection

The electro homoeopathic medicines Scrofoloso 5(S5) (Fig. 3) and Livome (Fig. 4) were purchased from the Biome Spagyric Pvt. Ltd, No.9 Sai Prateek Bazar, Kailash Nagar, Bhilai, Chhattisgarh, India. The batch number of the Scrofoloso 5 was 08/SPG/S5/13 and manufacture licence number:1134/J.S.V./2012. The batch number of the Livome was 10/liv/d/13 and manufacture licence number:1134/JSV/2012. The standard drug silymarin with the batch no SIST-019 and manufacturing licence no : KTK/25/575/2010 of the micro labs purchased from the local medical shop. The sample specimens were kept in our laboratory.

2.1.2 Chemicals

Carbon tetra chloride (CCl_4) was Purchased from the Nice Chemicals pvt. Ltd. Cochin-682024 India Pvt. Ltd., (Mumbai, India). All other chemicals and reagents used were of analytical grade.

2.1.3 Animals

Swiss albino female mice weighing 20-25 g and Male Wistar albino rats weighing 150-200 g were procured from S.S. Institute of Medical Sciences and Research Davanagere, Karnataka, India, and maintained at standard housing condition in Central Animal House, National College of Pharmacy, Shivamogga and were maintained. The animals were fed with commercial diet (Pranav Agro Industries Ltd., Sangli) and water ad- Libitum during the experiment. The Institutional Animal Ethical Committee (Reg.No.144/NCP/IAEC/CLEAR/08/2013-14) permitted the study.

2.1.4 Dosage

Doses of drugs were calculated to precisely match with the human doses employed according to the manufacturer's instructions. Standard drug silymarin (Micro Labs Ltd, Banglore) 14mg/kg/day, per oral (p.o.), i.e 2ml /kg/day p.o was administered. The dosage of the drug S5 and Livome is 0.5ml/day orally for rats of 150g to 200g b.wt.

2.2 Evaluation of Acute Toxicity

Experiments were performed using healthy young adult female Swiss albino mice, nulliparous, non-pregnant and weighing 20-25 g. Female mice were chosen because of their greater sensitivity to treatment. Acute oral toxicity test was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 423(2011) [3]. The animals were divided into six groups each containing six mice. They were identified by the markings using a yellow stain. One mouse was unmarked and the others were marked on head, body, tail, head and body, body and tail, to ease the observation.

2.2.1 Housing and diet

The animals were housed in polypropylene cages in a temperature controlled environment (23±2℃). Lighting was controlled to supply 12 h of light and 12 h of dark for each 24-h period. Each cage was identified by a card. This card stated the cage number, number and weight of the animals it contained, test substance code, administration route and dose level. The animals were fed with standard laboratory animal food pellets with water *ad libitum*.

2.2.2 Mode of administration

The test substance was administered in a single dose by using specially designed mice oral needle. Animals were fasted 3 h prior to dosing (only food was withheld for 3 h but not water).

2.2.3 Administration dose

After the fasting period maximum dose up to 1ml administered for mice of body weight 20-25gms. The test sample S5 was administered orally at a dose of 0.25, 0.5 and 1.0 ml per day for three groups and Livome was administered orally at a dose of 0.25, 0.5 and 1.0 ml per day for other three groups. After the administration of test samples, food for the mice was withheld for two hours.

2.2.4 Observation period

Animals were observed individually during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. All the animals were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioral changes.

2.2.5 Signs recorded during acute toxicity studies

Direct observation parameters include tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern are the other parameters observed. The time of death, if any, was recorded. After administration of the test substances, food was withheld for further 1-2 h. The number of survivors was noted after 24 h and then these were maintained for a further 14 days with a daily observation [4].

2.3 Evaluation of Hepatoprotective Activity

The Male Wistar albino rats were divided into 5 groups of 6 each. The animals group I (Normal Control) received no treatment but received food and water as the other group received. Carbon tetrachloride with olive oil (1:1) was administered to all the animals of groups II to V in the dose of 0.1 ml/kg/day, ip for 14 days. The group III animals were treated with the standard drug silymarin (Micro Labs Ltd, Banglore; 14mg/kg/day, po, i.e 2ml /kg/day po). The animals of group IV received S5 (1.0ml/kg/day, po) and the animals of group V received the Livome test sample (1.0ml/kg/day, po). The drugs were administered concomitantly for 14 days. The animals of all the groups were sacrificed on 14^{th} day under light ether anesthesia. The blood sample of each animal was collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37℃. The clear serum was separated by centrifugation at 2500 rpm for 10 min and subjected to biochemical investigation viz., total bilirubin [5], total protein [6], serum urea, serum alanine transaminase, aspartate transaminase, [7], and alkaline phosphatase [8].

2.3.1 Statistical analysis

Results of biochemical estimations were expressed as mean±SE of six animals in each group. The statistical analysis was carried out using one way ezANOVA. The difference in values at $P \leq 0.05$ was considered as statistically significant.

2.3.2 Study of histopathology

The liver samples were excised from the animals of each group after draining the blood and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin for 48 hrs. They were processed for paraffin embedding. The sections were taken at 5 μm thickness, processed in alcohol-xylene series and were stained with alum-haematoxylin and eosin [9]. The sections were examined microscopically for the evaluation of histological changes.

3. RESULTS

The results of the acute toxicity study indicated that there was no toxicity observed up to 1ml/day oral administration for mice of 20-25g b.wt. and up to 3ml for rats of 150-200g b.wt. for both S5 and Livome. After 14 days treatment, biochemical analysis of blood samples of CCl_4 treated animals showed significant increase in the levels of total bilirubin (2.32 fold), alanine transaminase (ALT) or serum glutamic-pyruvic transaminase (SGPT). (6.71 fold), aspartate transaminase (AST) or serum glutamic oxaloacetic transaminase (SGOT). (4.07fold) and alkaline phosphatase (ALP) (2.08 fold) as compared to the controls. In addition, the total protein level (39.63%), and urea level (38.63%) was decreased reflecting the liver injury due to the toxic effect of CCl_4. [10,11,12,13] have also reported the increase in SGOT, SGPT, sAcid phosphatase, ALP, urea and Creatinine due to hepatotoxicity induced by various toxicants in different experimental studies. The blood samples of the animals treated with the S5 and Livome were showed significant reduction in the levels of liver function serum markers and bilirubin and increased the total protein, and urea level to restore the normal condition. The effect was more pronounced in the animals treated with Livome is more effective than the effect of the standard drug silymarin (Table 1). The histological profile of control animal showed normal hepatocytes (Fig. 1a). The section of liver of the animals treated with CCl_4 exhibited intense centrilobular necrosis, vacuolization and macro vesicular fatty changes (Fig. 1b). The liver sections of silymarin treated animals showed normal hepatic architecture (Fig. 1c). Significant accumulation of fatty lobules was observed in the liver sections (Fig. 2d) of S5 treated animals. The liver sections of the animals treated with Livome exhibited significant liver protection against CCl_4 induced liver damage as evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration (Fig. 2e).

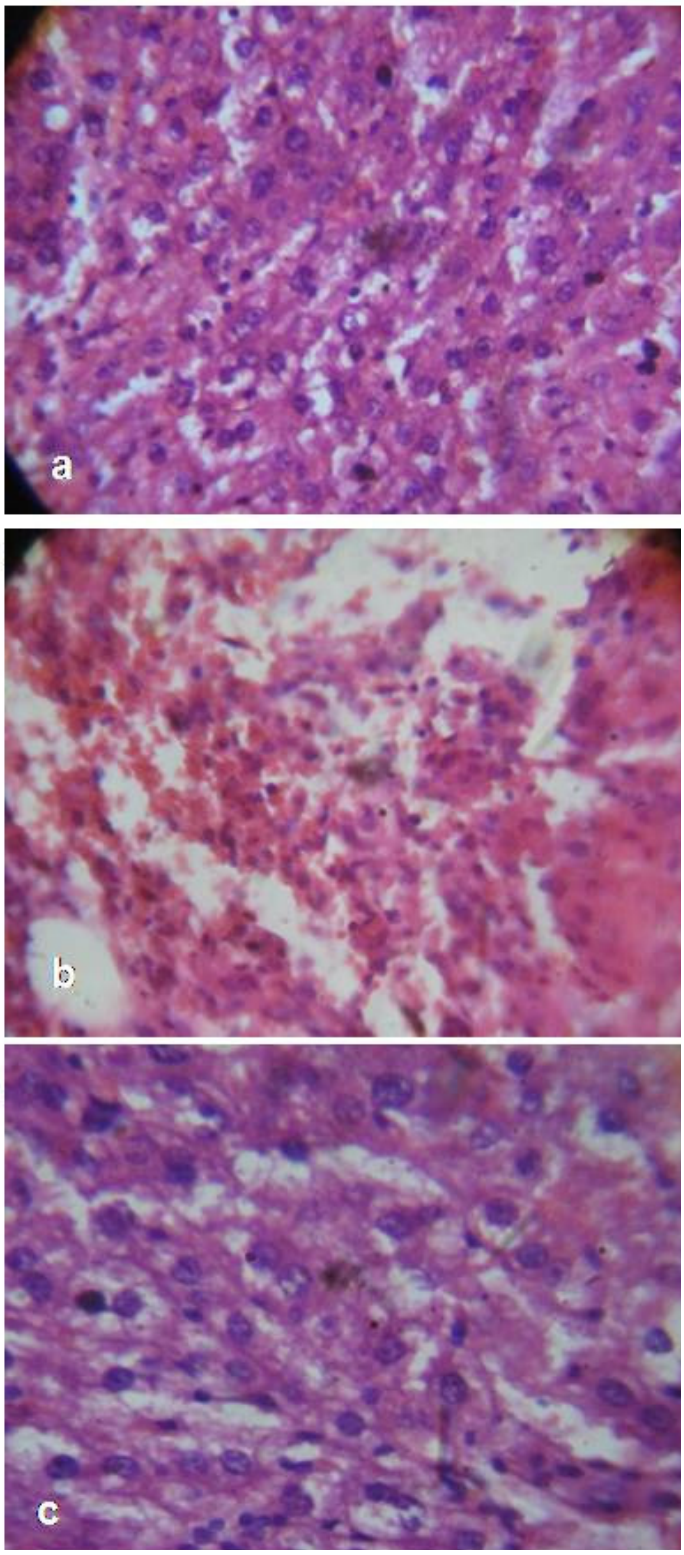

Fig. 1. Histology of liver tissues
a. Normal control, b. Ccl_4 treted, c.Standard control

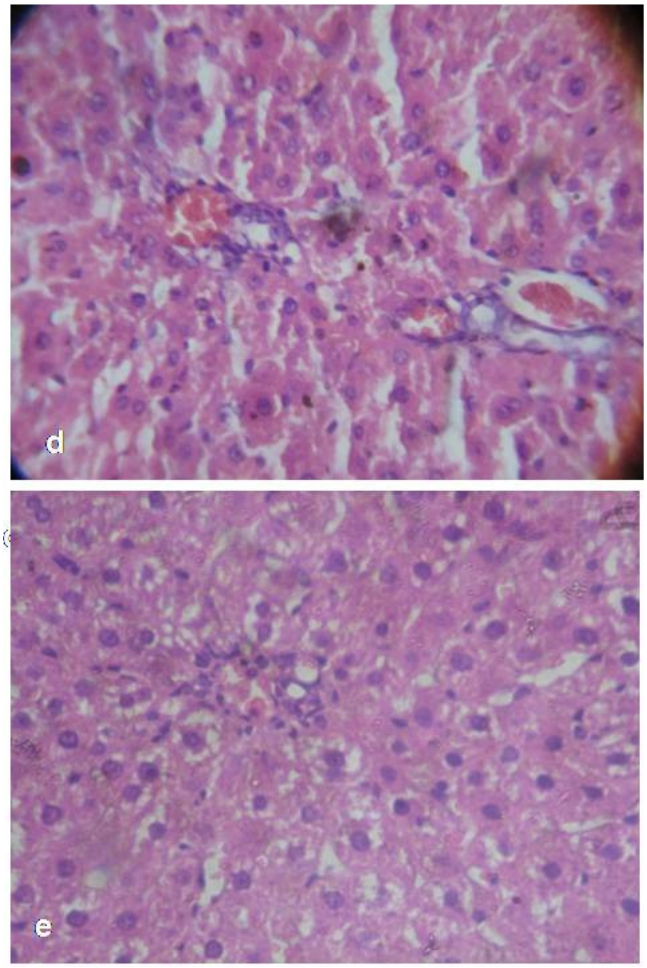

Fig. 2. Histology of liver tissue
d. With s5 e. Treated with livome

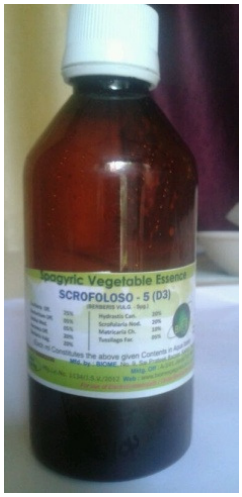

Fig. 3. Sample of scrofoloso-5

Fig 4. Sample of Livome

Table 1. Hepatoprotective effect of S5 and Livome by the restoration of serum biochemical parameters

Sl.no		Normal	CCl_4	Silymarin+CCl_4	S5 +CCl_4	LIVOME+CCl_4
1	Total bilirubin mg/dl Mean±SE	0.37±0.03	0.86±0.04	0.64±0.03	0.71±0.04**	0.57±0.04**
2	Total protein	8.25±0.13	4.98±0.27**	7.19±0.16	6.76± 0.19**	7.76±0.16**
3	ALT/ SGPT IU/L	150.67±6.98	1007.33±24.48**	347.50±11.75	428.67±31.72**	295.33±23.73**
4	AST/ SGOT IU/L	354.17±19.79	1443.83±74.09**	776.17±35.87	727.33±43.89**	673.33±30.81**
5	ALP IU/L	345.67±19.43	720.50±26.46**	569.00±26.43	565.67±54.37**	538.67±24.82**
6	UREA IU/L	77.83±3.93	130.33±5.90**	113.17±5.74	114.50±4.21**	107.67±6.52**

*Pairwise comparisons [Q=TukeyHSD: *=p<0.05 **=p<0.01] by using ezANOVA statistical analysis software*
Values are mean ± S.E.M; n=6. P<0.001 vs. Group CCl_4, P<0.01 when compared to control

4. DISCUSSION

Herbal medicine is gaining popularity in developing countries. Herbal based remedies are often believed to be harmless because they are "natural," and are commonly used for self-medication without supervision. This increase in popularity and the scarcity of scientific studies on their safety and efficacy have raised concerns regarding toxicity and adverse effects of these remedies [14]. These products contain bioactive principles with the potential to cause adverse effects [15]. In India there are large numbers of electro homoeopathic practitioners exist and millions of publics are receiving benefits from the electro homoeopathic mode of treatment for acute and chronic diseases [16]. There are number of electro homoeopathic spagiric préparations and products are avalable in the market. Our survey revealed that Scrofoloso 5 and Livome preparations are using to cure joundice patients by electropathic practioners more effectively. The electro homoeopathic medicine Scrofoloso 5 and Livome were prepared by using spagiric essences of multiple plants by cohobation technique. The phytochemical constituents and their chemical structures of the S5 and Livome preparations and their mechanism of action still need to further research. Hence in the présent investigation these two hepatoprotective electro homoeopathic herbal preparations Scrofoloso-5 and Livome obtained from the market were evaluated accute toxicity and hepatoprotective activity for scientific validation.

For accute toxicity studies we used adult non-pregnant female mice because they are physiologically and immunologically more sensitive than the male mice and also than the albino rats. There were no abnormal behaviour and toxicity observed up to 1ml of oral administration per day. This indicates the samples S5 and Livome have no toxicity. Lower dose 0.25 and 0.5 ml of administration of test samples were not showed any toxicity so we tried to check more larger dose. For mice 1ml is a larger oral dose. Even for such high dose administration of test sample S5 and Livome did not show any toxicity.

For hepatoprotective activity male rats were used since they exhibit more immunological resistance than the mice. The carban tetra chloride (CCl_4) is most common of the halogenated aliphatic hydrocarbons and is widely used in industry, laboratory and in the home as a cleaner and as fat solvent or degreaser. Clinically the absorption of CCl_4 causes dizziness, confusion and headache that can deepen coma, convulsions respiratory failure or be complicated by cardiac arrhythmia [17]. Carbon tetrachloride is one of the most commonly used direct intrinsic hepatotoxins to induce hepatic damage in the experimental study of liver diseases [18,19]. Administration of CCl_4 causes severe injury in rat's liver. This damage is recognized by an increase in levels of the total bilirubin, hepatic enzymes (AST and ALT) and ALP in blood serum, which are the indices of liver damage [20,21]. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloromethyl radical [22]. These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, which in turn give

products like malondialdehyde (MDA) that cause damage to the membrane. This lipid peroxidative degradation of biomembranes is one of the principal causes of hepatotoxicity of CCl_4 [23,24].This is evidenced by an elevation in the serum marker enzymes namely SGPT, SGOT and ALP [25]. There are numerous reports found in the literature concerned with the evaluation of the hepatoprotective activity of various natural and synthetic products including plant crude extracts and isolated phytoconstituents aginst CCl_4 induced hepatotoxicity. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin [26].

In the present investigation, the development of toxin-induced necrotic liver injury was noticed in rats by the administration of the toxin CCl_4. The results of biochemical parameters revealed the elevation of enzyme level in CCl_4-treated group, indicating that CCl_4 induces damage to the liver (Table 1). A significant reduction was observed in SGPT, SGOT, ALP, total bilirubin and increase in protein level in the groups treated with standard drug silymarin and test samples S5 and Livome. The enzyme levels were almost restored to the normal in Livome-treated group.

5. CONCLUSION

The results of our investigation revealed that the poly herbal electro homoeopathic preparations Scrofoloso 5 and Livome do not exhibit any toxicity and are more effective hepatoprotective than the standard drug as manifested by restoration of CCl_4 induced liver toxicity. More scientific research is requièred in understanding basic principles, phytoconstituents of active principle, and mechanism of action of electro homoeopathic herbal medicines by the application of current advanced techniques and tools for the benifit of future générations.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that The Institutional Animal Ethical Committee, National College of Pharmacy, Shivamogga, Karnataka India, approved all experiments and have been examined by the appropriate ethics committee. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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