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Research Article

Assessment of Acute Oral Toxicity of Synergistic Formulation Extract of Traditional Contraceptive Plants

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ABSTRACT

Some plants are used in folk medicine for prevention of pregnancy. The study aimed to assess the acute toxicity of synergistic formulation of traditional contraceptive plants. Acute toxicity means adverse change occurring immediately on exposure to a substance. Acute toxicity of crude petroleum and aqueous extract was evaluated by oral route administration to female mice by using OECD-423 guidelines. It was a single high extract dose of 2000mg/kg administered and the effects on mortality, behavioral pattern as well as spontaneous locomotors activity were evaluated. The limit dose of 2000 mg/kg did not cause any mortality. The findings suggested that the LD50 value of aqueous and petroleum ether was found to be greater than 2000 mg/kg. Hence, synergistic extract of plants is nontoxic. This work is useful to find out the dose structure for further experimental work on evaluation of traditional contraceptive plants and their activity.

Keywords: Contraceptive activity, Acute Toxicity.

INTRODUCTION

From ancient time, people searched for cures for his disease from nature. This ancient tradition becomes traditional medicine practice being the first point of healthcare for many people around the world. There has been an increased focus on medicinal plant research and a large amount of evidence has been collected to show its immense potential in various traditional health systems. Plants have been extensively used as medicines since a thousand years ago and the use of herbal products should be based on scientific origin in order to make sure the plants are safe to consume.

In the last few years, studies have been carried out on a large number of plants, used by traditional practitioners for centuries[1]. The products from the medicinal plants have become universally popular in primary healthcare, and some have been regarded as readily safe because of natural source. This presumption has led to plant products being widely used as self-medication without compromising health effects[2]. Polyherbal formulations are widely used for the health of mankind mentioned in the study of Karandikar[3]. Herbal drugs, singularly and/or in combinations, contain a myriad of compounds in complex matrices in which no single active constituent is responsible for the overall efficacy[4]. Large number of plant species has been identified through ethnobotanical and ethnopharmacological studies as potential sources of therapeutic agents and pure products. Whole plants or parts of them are prepared and administered as oral decoctions, steam baths, infusion, or enemas. Most remedies are a mixture of various ingredients of two or more plant species that work in synerg[5].

The traditional contraceptive plants *Ricinus communis* L., *Moringa oleifera* Lam., *Sapindus emarginatus* Vahl. *Crotalaria juncea* L., *Trigonella foenum-graecum* L. are used in folk medicine as alcoholic and aqueous preparations for prevention of pregnancy[6]. The acute toxicity study was performed to ensure the safety and for the determination of lethal dose (LD50) value of plant extract. "LD50" is the amount of a given substance required to kill 50% of a test population (lab rats or other animals). Acute Toxic Class Method is a stepwise procedure, where three animals of the same sex per fixed dose level are used. Dependent on the outcome, a decision is made as to whether further testing is necessary[7].

In the present study the synergistic petroleum and aqueous extract of *Ricinus communis* L., *Moringa oleifera* Lam., *Sapindus emarginatus* Vahl. *Crotalaria juncea* L., *Trigonella foenum-graecum* L. was subjected to oral acute toxicity. Acute oral toxicity study was conducted in female mice as per Organization for Economic Cooperation and Development (OECD) guideline[8].

MATERIAL AND METHODS

Collection of Plant Material

Fresh Seeds of *Ricinus communis* L., *Moringa oleifera* Lam., *Sapindus emarginatus* Vahl. *Crotalaria juncea* L., *Trigonella foenum-graecum* L. were harvested from their natural habitat. Plant Samples were collected from various areas of Ahmednagar District, India and were identified from Botanical Survey of India, Western Circle, Pune. The voucher specimens were deposited in the Herbarium, BSI, Pune as well as Herbarium of Department of Botany, New

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Arts, Commerce and Science College, Ahmednagar. (ABK 001,ABK 002,ABK 003,ABK 004,ABK 005).

Extraction of Plant Material

Collected seeds were cleaned, dried, and finely powdered in a grinding machine. 250 gm of each seed samples were powdered and was extracted in Petroleum ether using Soxhlet extraction or hot continuous extraction[9,10]. In this method, finely ground sample was placed in a porous bag and placed in thimble chamber of the Soxhlet apparatus. Petroleum ether solvent was heated in the bottom flask, vaporizes into the sample thimble, and condenses in the condenser and drip back. It is continuous process. Once the process has finished, the solvent was evaporated using a rotary evaporator, leaving a small yield of extracted plant material (about 10 to 15 ml) in the glass bottom flask. The advantage of this method is that large amounts of drug can be extracted with a much smaller quantity of solvent. The extract was preserved at 4^0 C till the acute oral treatment. Aqueous extract was prepared at the time of dosing the animals. It was prepared with distilled water. Powdered seed samples were extracted separately.

Synergistic Formulation

Aqueous extracts of selected plants seeds were mixed together in 1:1:1:1:1 ratio. Similarly, Petroleum ether extracts were also mixed together in equal quantity and it was formulated in the same way by mixing together.

Ethical Approval

Authors hereby declared that the experimental protocol was approved by the Institutional Animal Ethics committee (RP31/1516). The work was carried out at Apt Research Foundation, Pune. Each animal was used only once. For ethical reason, all animals were sacrificed at the end of the study. Experimental protocol was followed according to Guidelines for Care and Use of Laboratory Animals. All rules were followed as well as specific national laws where applicable.

Experimental Animals

Female mice (4-6 week old) were selected as per the OECD guidelines[8]. Female mice were selected because literature surveys of conventional LD50 test shows that usually there is little difference in sensitivity between sexes, both in those cases where differences are observed, generally females are found slightly more sensitive[11]. The animals were housed in a well-ventilated animal house with 12 h/12h light /dark cycle. The animals were randomly selected, marked to permit, individual identification, and kept in their cages for at least 5-7 days prior to dosing to allow for acclimatization to laboratory conditions.

Acute toxicity studies

Experimental procedure and handling of animals was carried out according to the OECD-423 testing guidelines. Minimum animal number was taken to estimate the acute oral toxicity of synergistic formulation. The experiment was performed in a stepwise manner which involved 12 animals for step 1 and step 2. Since the herbal dose supposed to be safe, the higher dose of 2000 mg/kg was given.

In Step I three female mice were administered by oral route with the synergistic aqueous extract at 2000mg/kg dose and another 3 animals administered with pet ether extract at 2000mg/kg dose (200mg of extract dissolved in 1 ml of water and dosed as per body weight. 0.2 ml was administered per mouse with average weight of 20 gm). The animals were deprived of feed 4 hour prior of dosing and 2 hours after dosing. After the extracts administration, feed but not water was withheld for 3-4 hours. The animals were monitored for 14 days. Mortality rate and body weight were recorded weekly. Animals were under observations continuously individually at least once and periodically during the first 24 hours (with special attention given during the first 4 hours), for a total of 14 days. For Step 2, Another 3 female mice were administered the aqueous extract at 2000mg/kg dose and 3 mice administered with pet ether extract at 2000mg/kg dose (3 females for each group) seven days after step 1. The same observations were repeated for these animals.

All observations were systematically recorded with individual records being maintained for each animal. The information is useful to determine the relevance of the test for the protection of human health and the environment and will help in the selection of an appropriate starting dose.

Visual Observation

Additional observations were necessary if the animals continue to display signs of toxicity. Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems and behaviour pattern. Attention was given to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. When animal was found dead, the time of death was recorded as precisely as possible. All signs of ill health, together with any behavioral changes or reaction to treatment were recorded for individual animals.

Physical examination of the animals was performed prior to commencement of treatment to ensure that the animals are in good state of health. The mice were closely observed for any indications of toxicity effect within the first eight hours after the treatment period and daily further for a period of 14 days. Surviving animals were weighed after 7day duration and visual observations for mortality, behavioral pattern changes such as weakness, aggressiveness, food or water refusal, diarrhea, salivation, discharge from eyes and ears, noisy breathing, changes in locomotors activity. Coma, injury pain or any signs of illness in each treated group were monitored carefully on daily basis throughout the experiment period. In this study no deaths were reported in first step and second step of petroleum ether extract but in aqueous extract, one female mice was dead during second step. The present study, suggesting that the LD50 value of aqueous and petroleum ether was found to be greater than 2000 mg/kg.

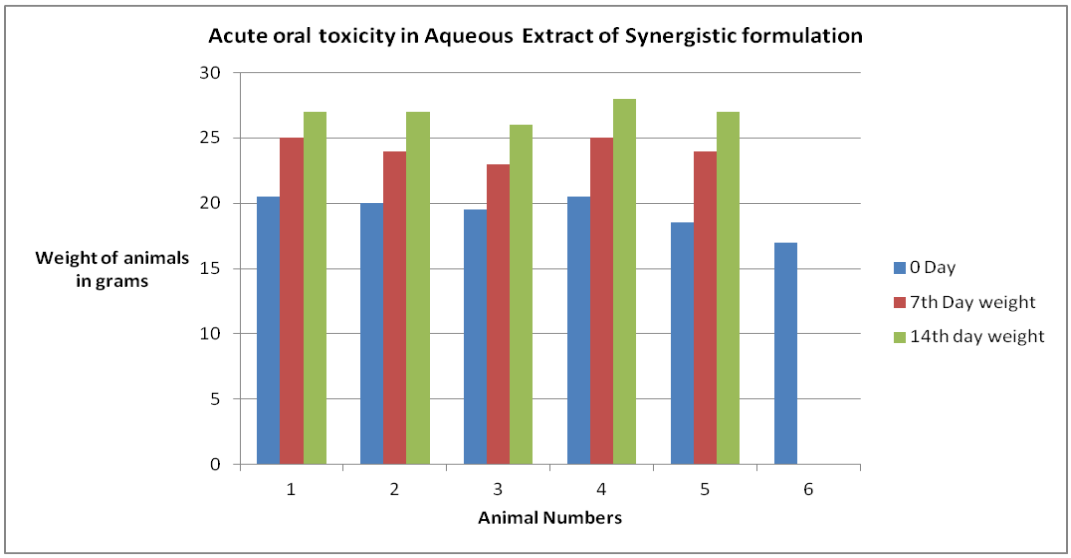
Individual weight of animals was determined shortly before the test substance was administered and at least weekly thereafter. Change in Weight was calculated and recorded. At the end of the test surviving animals were weighed.

RESULT

Table 1: Acute oral toxicity in Aqueous Extract of Synergistic formulation.

Aq. Ext	Animal No.	Sex	Body weight data in g. Day 0	Day 7	Difference in Wt. day 7	Day 14	Difference in Wt. Day 14
Step 1	1	F	20.5	25.0	4.5	27.0	6.5
	2	F	20.0	24.0	4.0	27.0	7.0
	3	F	19.5	23.0	3.5	26.0	6.5
	Mean		20.0	24.0	4.0	26.7	6.7
	SD		0.5	1.0	0.5	0.6	0.3
Step 2	4	F	20.5	25.0	4.5	28.0	7.5
	5	F	18.5	24.0	5.5	27.0	8.5
	6	F	17.0	Death			
	Mean		18.7	24.5	5.0	27.5	8.0
	SD		1.8	0.7	0.7	0.7	0.7

The LD50 value of all the extracts was found to be greater than 2000 mg/kg



Acute toxicity

The acute oral toxicity study was conducted as per the OECD guidelines 423, where the limit test dose of 2000 mg/kg was used. The synergistic aqueous and petroleum ether extracts were administered one time to a group of three female mice up to 14 days. Within same days there was no mortality in the mice treated with aqueous as well as petroleum ether extracts. Therefore, the same treatment of synergistic extracts was given to be another three mice. In the present work no deaths were reported in first and second step of petroleum ether extract but it was found that during second step of aqueous extract one of the mice was dead. Otherwise in both steps all the mice were active with good breathing activities. There was no any significant change in locomotors activity. The calculated values suggesting that the LD50 value of aqueous and petroleum ether was found to be greater than 2000 mg/kg.

Individual weights of animals were determined shortly before the test substance was administered and at least weekly thereafter. Weight changes was calculated and recorded. At the end of the test surviving animals were weighed.

Throughout 14days of observation there were no significant changes recorded in behavior in any of the animals such as hyperactivity, morbidity, apathy etc. In physical observations there was neither weakness, aggressiveness nor any symptoms of diarrhea, discharge from eyes and ears etc. No abnormal changes attributable to treatment were noticed in body weights and treatment related changes like respiration rate and heart rate.

There were no clinical signs of toxicity or treatment related mortality during the observation period among the mice. The animals appeared very healthy and their physical activity appeared normal.

Body weight

Acute oral toxicity was measured by considering one of the important body weight parameter. The body weights were recorded on test day 1 (pre-administration fasting weight) and on days 7^{th} and 14^{th} post treatment.

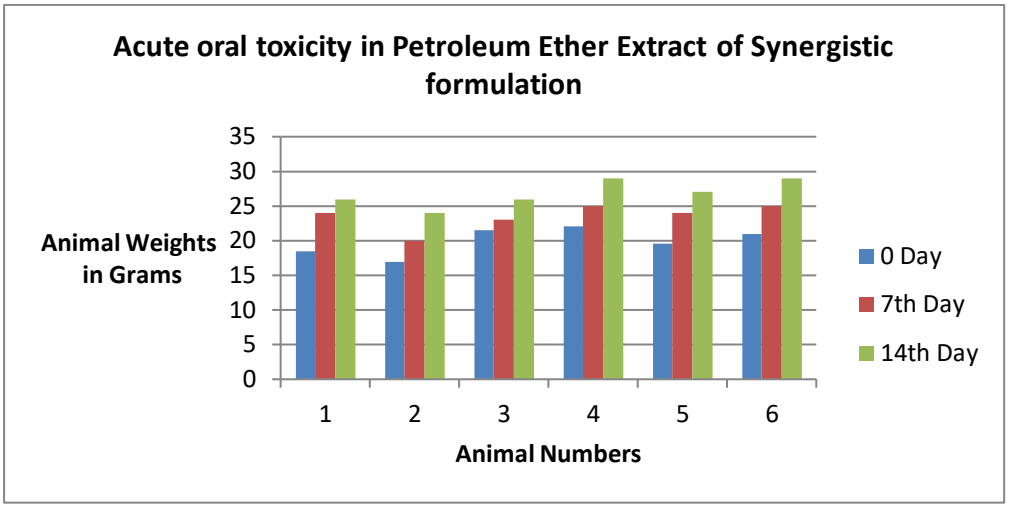
The body weight data is depicted in table no.1 and 2. The results showed that the acute treatment with crude aqueous and petroleum ether extracts of synergistic formulations by oral route at 2000 mg/kg did not cause death in female mice for 7 or 14 days during observation time, expect one case of mouse.

The body weight was measured from 0-7 and 7-14 days of treatment. In step-I of aqueous extract, the body weight was continuously increasing from mean 20.0 ± 0.5 to 26.7 ± 0.6 gm. It showed that in first step of treatment no

Table 2: Acute oral toxicity in Petroleum Ether Extract of Synergistic formulation.

			Body weight data in g.				
Pet. Ether	Animal No.	Sex	Day 0	Day 7	Difference in Wt. day 7	Day 14	Difference in Wt. Day 14
Step 1	1	F	18.5	24.0	5.5	26.0	7.5
	2	F	17.0	20.0	3.0	24.0	7.0
	3	F	21.5	23.0	1.5	26.0	4.5
	Mean		19.0	22.3	3.3	25.3	6.3
	SD		2.3	2.1	2.0	1.2	1.6
Step 2	4	F	22.0	25.0	3.0	29.0	7.0
	5	F	19.5	24.0	4.5	27.0	7.5
	6	F	21.0	25.0	4.0	29.0	8.0
	Mean		20.8	24.7	3.8	28.3	7.5
	SD		1.3	0.6	0.8	1.2	0.5

The LD50 value of all the extracts was found to be greater than 2000 mg/kg.



toxicity was observed. As there was no mortality, the second step was carried out as per the OECD guidelines.

The similar treatment was given to another three female mice having similar body features. It was considered as step-II. In second step, body weight of individual mice was also increased from 18.7 ± 1.8 to 27.5 ± 0.7 gm. One of the mice was dead, but it is negligible as per OECD-423 guideline in a limit test.

The assessment of acute oral toxicity was also studied by giving the treatment of petroleum ether extract. The synergistic formulation was extracted by using petroleum ether. The treatment to the mice was given in similar way like aqueous extract. In the first step of treatment with 2000mg/kg, the body weight of mice was increased rapidly up to 7 days and then it was in the similar manner up to 14 days. The weight was increased from 19 ± 2.3 to 25.3 ± 1.2 gm. It showed that, after treatment the mean body weight was increased by 6.3 gm per individual mice (Graph No. 2).

There was no death in Step-I, hence similar treatment was followed for another group of three mice which was considered as Step-II. Similar data on body weight of female mice was obtained in step-II also. As per the values depicted in table No. 1 the mice were healthy.

There were no toxicological significant changes in body weight of animals treated with selected plant extracts of aqueous and petroleum ether. The LD50 value of all the extracts was found to be greater than 2000 mg/kg.

DISCUSSION

Acute toxicity is usually defined as the adverse change occurring immediately or a short time following a single or short period of exposure to a substance or substances[12]. The objectives of oral toxicity tests are to identify the dose causing major adverse effects and also to estimate a minimum dose for the substance or material lethality.

The present work on acute toxicity was observed by using the synergistic formulation. If the individual plants were referred for acute toxicity, the reports are variable. The species of *Crotalaria* grow abundantly in tropical and subtropical zones and are popular medicine[13,14]. These plants are rich in pyrrolizidine alkaloids (PAs), which are the main toxins derived from plants that are transferred to humans and animals[14,15]. Ricinus communis L. is a large red and green leaved which has the therapeutic effect. The castor seed contains about 40% oil, 1–5% ricin, and 0.3–0.8% ricinine. From the toxicity studies of *Ricinus* seed extract data revealed that, all the synthesized compounds proved to be non-toxic at tested dose levels and well tolerated by the experimental animals as there LD50 cut of values > 2000 mg/kg[16]. At the desired dose it did not show any toxic effect[17]. Safety evaluation studies of *Moringa oleifera* Lam. showed that ethanolic and aqueous extract

of both fruit and leaf was well tolerated by experimental animals. The ethanolic extract of fruit showed highest phenolic content, strong reducing power and free radical scavenging capacity. Safety evaluation studies showed no toxicity of the extracts up to a dose of 100 mg/kg body weight[18]. It has shown that the extracts of *Sapindus mukorossi* have a protective capacity but not toxic both *in vitro* and *in vivo*[19]. The saponin fraction of *Sapindus mukorossi* has a protective capability both in vitro on primary hepatocyte cultures and in vivo in a rat model of CCl4-mediated liver injury[20]. Further they have suggested that the inclusion of *Sapindus mukorossi* fruit pericarp in the management of liver disorders is justified. The mice treated with *Sapindus trifoliatus* at a dose of 2000 mg/kg body weight, exhibited normal behaviour without any sign of passivity[21].

The acute toxicity of *Trigonella foenum-graecum* was carried out and suggested the safe doses and identified the toxicity effects of the fenugreek seed extract[22,23]. The acute oral treatment of mice with the crude aqueous and petroleum ether extracts of synergistic formulations did not show any toxic effect. The findings were based on the physical observations. There were no significant changes in weakness, aggressiveness, food or water refusal, symptoms of diarrhea, salivation, discharge from eyes and ears.

In the present study, our results are very much significant when compared with the previous literature. It was found that no deaths were reported in first and second step of petroleum ether extract but one of the animals was found dead in aqueous extract in second. So, findings suggested that the LD50 value of aqueous and petroleum ether was found to be greater than 2000 mg/kg. Hence, synergistic extract of plants is nontoxic in acute dose of 2000mg/kg. The limit doses of 2000 mg/kg did not cause any mortality or signs of acute toxicity in the mice tested during the observation period. The synergistically active compound provides greater therapeutic benefit than the individual ingredients alone, or the combined ingredients in different concentration ranges. It showed that neither individual plant species nor synergistic extracts has acute oral toxicity at higher doses of extracts.

This experimental work can be used for further treatment of plant extract against the illness or particular diseases. The present assessment was used for authentication of traditional contraceptive plants.

CONCLUSION

The results showed that synergistic formulation extract of plants is nontoxic with acute dose of 2000mg /kg. These plants *Ricinus communis* L., *Moringa oleifera* Lam., *Sapindus emarginatus* Vahl. *Crotalaria juncea* L., *Trigonella foenum-graecum* L. are medicinally important referred by many herbalists. Hence it can be safety and useful for the determination of lethal dose (LD50) value of plant extract in further animal experiment for study of contraceptive activity. The desired dose of 2000 mg/kg of the synergistic extract made from the studied above plants is very much secured, safety for clinical trial also.

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