https://ijrps.com

ISSN: 0975-7538
Research Article

Acute oral toxicity studies of isolated compounds in *Lawsonia inermis* and *Leptadenia reticulata* in swiss albino mice

Rajeswari J*, Rani S

Annamalai University, Cuddalore District, Chidhambaram, Tamil Nadu, India

ABSTRACT

The compounds which is isolated from *Leptadenia reticulata* and *Lawsonia inermis* is sample 1 (6-amino-3-hydroxy-4-(4'-methylphenyl)-2*H*chromen-2-on) and -sample 2 (3, 7, 4', 5'-Tetrahydroxy-6- methoxyflavone).These compounds were undergone acute toxicity studies for dose fixation. In acute toxicity studies feminine (8-week old) young Swiss albino mice 20-25 g weight is used. The animals are grouped and in each group 3 animals are used. Generally acute toxicity study is the preliminary study for conducting animal experiments, it is mainly done for dose fixation. The dose fixation is mainly categorized for safer doses. The minor changes and variations in sexes will play a role in animal studies, but the feminine will be typically adoptable for this animal experiments because it is more sensitive than males. After the toxicity study is done the LD_{50} value is calculated by having a note on mortality determination. The $LD_{50\ value}$ which is calculated as 200mg/kg and gross behavioural studies like respiratory parameters, salivary secretions, sense of touch and sound, writhing reflex, tremor, convulsions, hind limb paralysis, GIT motility and induction of diarrhea were explained. The acute toxicity were done according to OECD Guidelines 423.

Keywords: *Leptadenia reticulate; Lawsonia inermis;* 6-amino-3-hydroxy-4-(4'-methylphenyl)-2*H*chromen-2-on; 3, 7, 4', 5'-Tetrahydroxy-6- methoxyflavone.

INTRODUCTION

Toxicology' in terms of habitually referred to be known as knowledge towards toxicity and the knowledge started in historic days itself, and it's recognized as hepatotoxic flora and fauna. It has been used for their juices (extracts) for looking out. Then it also added with procedure for maintaining the protection for a selected complex. In Time, medicine (hepatotoxicology) might even be printed as a result of to revise the dangerous / toxic things of medication. It also includes completely different synthetic substance with pressure on discovery, interference and handling of toxic substances. When the total data regarding destructive effect of a substance is known from that the amount of the quantity has been determined for its harmless usage, typically this can be often referred as its compound (grade) (Tripathi.,2008).

Usually the Pharma companies will undergo certain research for determining the toxicity levels. The drugs will passed through the toxicity levels in the order of a) acute b) Sub acute c) chronic. The companies will supply information regarding toxicity potential and it may develop on temporary revelation. These results are applied on various routes of administration. The information are influenced by many causes namely 1) perform as a result of the arrangement for cataloging and grouping. (2) Gives first data on the style of cytotoxic act (3) Facilitate a hit of a amount for the replacement complex.(4) Facilitate in quantity purpose in creature study (5) Facilitate verification of LD50 data that supply several index of probable varieties of drug action. The aim of the acute toxicity check is to work out the curative key. The larger the index, safer is that the substance. The factors that embody however not restricted in oral LD50 is (1) organization of creature(animals) (2) continuance of a slim vary of body weights (3) acceptable variety of creature per cluster (4) Recognition of creature check subject (5) Abstinence (6) acquisition of water (Shetty et al., 2007).

Each LD50 carried out for a chemical should embrace a minimum of 2 ways of revelation, typically oral and epithelial duct route. Reckoning on the character of a material, the route may be under the change for incorporating the test in gasp, skin or alternative choosy coverage. For marine substance, pharmacological medicines are conferred because the middle lethal absorption (LC50) the calculable absorption of ecological revelation leading to five hundredth transience of the populace of investigational creatures.

* Corresponding Author
Email: sasswathi86@gmail.com
Contact: +91-
Received on: 07-09-2016
Revised on: 23-09-2016
Accepted on: 27-09-2016

Each LD50 results shows the lethal dose, it won`t influence the initial fatal nature of the compound and it does not recommend the sufficient data to reason a substance. It won`t give any action on varying mechanism of reaction in cyanogenetic substances. In Fact Comparisons are important only if substances are homologous and have an equivalent mechanisms of action. It has the influence in differentiating fatal substance for a specimen in an exceedingly specific species, time period and genders of creature (Frank Barlie., 2008).

Traditional and practice of medication is widely used within hindrance and curation of varied diseases. It has become popular by paying public interest for the long decade of twenty years as this type of drugs are certainly available in various places. (Humber., 2002). Plant-obtained foods, considerably vegetables and fruits are usually thought-about to be very helpful substances of the human food. They supply major significance in common place of living by giving wide variety of supplements and different substance that amplify the healing armory. Natural product involving in a major role inside the event of latest drug leads for the curing and hindrance of disease (Newman et al., 2003).

Lawsonia inermis is a vital medicative plant within the Indian system of drugs. It's normally known as henna that develops in hot regions. The fluid substance which is obtained from green coloured foliage of henna is employed to colour hands in involved styles. Therefore the major coloring substance is laws one, 2-hydroxy-1, 4-naphthoqunone (Prosen et al., 2005). Root is taken into account as an important drug for infection of her- pes. The root is used as an mordant used for sore eyes. The pulp root is used as a medicine for head boilers .The juice of the root of the together with ready indigo is used as a anti fertility agent and it also plays a major role in treatment of madness and anxious disorder (Chopra et al., 1956 ; Reddy 1988 ; Shivalinagappa et al., 2001).

Leptadenia reticulata (jivanti) is found in tropical and sub-tropical areas of Asia and Africa. It is commonly grown in Gujarat and then in, sub - chain of mountains tract from environmental region to geographical area and hills of khasi and entire dry land and its height is of, 900 metres. The other name of the plant is Jivanti to nicth at bit raise the healthy status of the body as mentioned in the older science. It's utilized for the persons who is suffering by energy less nature. In additionally it will increase long life, recollection of memory, immune modulation and implementation. (Chauhan et al., 2010). The three doshas that is (Vatta, tyrannid and Kapha), and it is normally used in weakness, controlling the uncontrolled release of semen, as a tonic and poison of snake.(Dandiya 1970; Bhatt et al.,2006) abortifacient, tonic, restorative, disinfectant, antifabrifuge, for curing the wound and in mouth lesion (Vaidya 1965).

MATERIALS AND METHODS

Place of compilation and authentification of plants

The entire plant of *Leptadenia reticulata* and the roots of *Lawsonia inermis* were procured from district of Thirunelveli in the state of Tamil Nadu, India. The plant materials were taxonomically known and approved by Dr. V. Chelladurai, (scientist C), Central council for analysis and research in Ayurveda and Siddha in Govt. of India.

Processing of plant samples

The samples are cleaned in distilled water. The rinsed samples were clean, shade dried and made as a powderby using mechanical grinder. The small -grained materials were kept in air tight synthetic resin luggage until use.

Preparation of Extracts

The dried components were extracted by using alcohol by using a apparatus of soxhlet extractor. (Vaidya 1965). The extract which is obtained is concentrated with rotary evaporator till dry powder was obtained. Isolation is done by column chromatography and the isolated compounds are characterized by IR, NMR, MASS spectrometry.

Acute oral toxicity study

The acute oral toxicity study (OECD 423) were done by inducing isolated compounds from *Leptadenia reticulata sample 1* (6-amino-3-hydroxy-4-(4'-methylphenyl)-2*H*chromen-2-on, mol formula is(C16H13NO3) and the Sample 2 which is obtained from isolated compound from *Lawsonia inermis (*3, 7, 4', 5'-Tetrahydroxy-6-methoxyflavone and the Molecular Formula deduced as (C16H12O7).

Selection of animals

The experiment were done by using feminine (8-week old) young Swiss albino mice 20-25 g weight. The current study was authorized by Institutional of animal committee (Ethical) of Kmch college of Pharmacy Coimbatore, (KMCRET/Ph.D/12/2013-2014). Feminine rats were choosen as a result of literature surveys, LD50 datas reveals, typically there will be very minor dissimilarity in nature of sense in sexes and however in this study wherever variations is found, feminines are typically a lot of responsive in nature. (Lipnick et al., 1995**).**

Maintenance of Animals

The mice were placed in properly numbered massive propylene cages with stainless-steel high grill having facilities for pelleted food. The animals were maintained in twelve hours lightweight and kept in darkness at 28oC ± 2o C in an exceedingly good maintained animal house below normal circumstances in massive propylene cages. These animals were acclimatize to normal lab conditions for ten days before the begin-

Table 1: Dose determination for animal group

Animal group	Dose of the compounds
Group 1	5mg/kg (Comp A)
Group 2	50mg/kg (Comp A)
Group 3	300mg/kg (Comp A)
Group 4	2000mg/kg (Comp A)
Group 5	5mg/kg (Comp B)
Group 6	50mg/kg (Comp B)
Group 7	300mg/kg (Comp B)
Group 8	2000mg/kg (comp B)

Table 2: Gross behavioural studies in rats on administration of extract of sample 1(6-amino-3-hydroxy-4-(4'-methylphenyl)-2Hchromen-2-on (Leptadenia reticulata) at the dose of 300 mg/kg

Observation	Up to 3hrs	3½hrs	4hrs	4½hrs	5hrs	5½hrs	6hrs	12hrs	24hrs
Gross activity	I	I	I	I	I	I	I	I	I
Respiration	II	II	II	II	II	II	II	IV	IV
Writhing	II	II	II	II	II	II	II	II	II
Tremor	II	II	II	II	II	II	II	II	II
Convulsions	II	II	II	II	II	II	II	II	II
Hind limb paralysis	II	II	II	II	II	II	II	II	II
Sense of touch and sound	II	II	II	II	II	III	III	IV	IV
Salivation	II	II	II	II	II	III	III	IV	IV
Diarrhoea	II	II	II	II	II	II	II	II	II
Mortality	II	II	II	II	II	II	II	II	2X

I – Positive, II - No effect, III- Slight effect , IV - strong effect(depression), X- Death

Table 3: Gross behavioural studies in rats on administration of extract of sample 2 (3, 7, 4', 5'-Tetrahydroxy-6- methoxyflavone (Lawsonia inermis) at the dose of 300mg/kg

Observation	Up to 3hrs	3½hrs	4hrs	4½hrs	5hrs	5½hrs	6hrs	12hrs	24hrs
Gross activity	I	I	I	I	I	I	I	I	I
Respiration	II	II	II	II	II	II	II	IV	IV
Writhing	II	II	II	II	II	II	II	II	II
Tremor	II	II	II	II	II	II	II	II	II
Convulsions	II	II	II	II	II	II	II	II	II
Hind limb paralysis	II	II	II	II	II	II	II	II	II
Sense of touch and sound	II	II	II	II	II	III	III	III	IV
Salivation	II	II	II	II	II	III	III	III	IV
Diarrhoea	II	II	II	II	II	II	II	II	II
Mortality	II	II	II	II	II	II	II	II	2X

I – Positive, II - No effect, III- Slight effect , IV - strong effect(depression), X- Death

ning of the experiment. The pelleted diet is given to the animals as a food which is purchased from Coimbatore (AVM Foods). Further animal studies, done by following ethical guidelines advised by the institutional animal ethics committee (IAEC). Husk bedding is made by paddy. The animals were given, unlimited of filtered drink.

Experimental Procedure

To determine the dose of isolated compound of *Lawsonia inermis* and *Leptadenia reticulata*, acute-toxicity studies were carried out. Each group contains 3 animals and will be housed individually. The test compounds had been given as a single dose, While giving the Subsequent dose of the test drugs, food should be withheld for 1 hr in mice. Animals will be observed for behavioral modification, toxicity checking for a time period up to 24hrs and observations will be done up to 14 days after acute toxicity dose. The compounds are given at the dose of levels five, fifty, three hundred and two thousand (2000) mg/kg body weight. If the I st group animals survived, the further group animals will receive a higher dose as per OECD Guidelines 423.

RESULTS AND DISCUSSION

The acute toxicity of sample 1 (6-amino-3-hydroxy-4-(4'-methylphenyl)-2*H*chromen-2-on) and sample 2 (3, 7, 4', 5'-Tetrahydroxy-6-methoxyflavone) was used in this experiment. The guidelines which is adopted for this study is OECD-423. It is determined for safe dose for administrating to animals. The reports of acute toxicity study confirms that LD_{50} data`s of both the sam-

ples 1 and 2 was high, in addition to these the animals monitered continuously for four hours intially, and then at an interval of two hours for 24 hours to examine any change occurs (or) observed in the rats behaviour such as respiration, writhing, CNS excitation, Weakness in muscles, reflexes, salivation, diarrhoea, food intake, depression and mortality. The observation of gross behavioural studies revealed that after administration of sample 1 and sample 2 showed respiratory parameters, salivary secretions, sense of touch and sound. The writhing reflex, tremor, convulsions and hind limb paralysis were absent. No alteration was found in GIT motility and induction of diarrhoea with these sample. This experiment reveals that LD_{50} value as 200 mg/kg**.** so, remedial dose is fixed as 1/10th (20 mg/kg of the mortal dose for the purpose of abortifacient investigations.

DISCUSSION

Phytotheraputic substances from nourishing plants became worldwide usable substance in primary aid, significantly in developing countries, and a few are incorrectly considered as secured simply and so these substances are attained from a nature. Yet, these herbal compounds from flora are probably secured wholely without any deviating effects in physical condition, and so it is largely worned as natural medicine. (Vaghasiya et al., 2011). However, there's an be short of approved methoical study regarding fatal and bad effect. So the systematic toxic study is very essential to establish the safer doses and then it reveal itself as a appropriate sign to find the curative key of heiling substances. (Rang et al., 2001).

In the gross behavioural studies When administered an acute toxicity dose of sample 1 (6-amino-3-hydroxy-4- (4'-methylphenyl)-2*H*chromen-2-on) in mice there was no decrease in the rate of respiration initially, but from 12^{th}hr onwards respiration was strongly depressed. Similarly in sample 2 (3, 7, 4', 5'-Tetrahydroxy-6- methoxyflavone) of there was no decrease in the rate of respiration up to 6^{th} hr and from 12^{th} hr onwards strong depression was observed. These samples acted on respiratory center through CNS causing a decrease in oxygen supply to lungs which leads to decrease in respiration.

Writhing-There is no writhing effects seen till 24 hrs.

Tremor-It is involuntary, rhythmic, contraction and relaxation involving oscillations or cramp movements of 1 or additional body components .But the samples did not produce any tremor even after 12 hr. It is inferred that samples have not acted directly on CNS.

Convulsion- The remedial stipulation occurs wherever body physique indenture and slow down fastly and it repeats for certain times as a result to the state of unrestrained trembling of the body. The samples did not produce effects on convulsion.

Hind limb paralysis- It is loss of muscle to perform for one or additional muscles. Dysfunction is a loss of feeling (sensory loss) within the affected space if there's sensory harm in addition as well as motor. The samples did not produce any effects in paralytic effect.

Sense of touch and sound- sensation of touch and sound by acting on ascending reticular activating system enteroceptive organs which receive stimuli from muscle and joints. In sample 1 shows the depressive effects in 12 to 24 hrs. In sample 2 shows the depressive effects in $5^{½}$ hrs to 24 hrs, but the strong depression was observed in 24 hrs.

Salivation- Drugs, acting directly on cholinergic or histaminic receptors may stimulate salivary secretions. In sample 1 shows effects in 12 to 24 hrs,in sample 2 the effects showed in $5^{1/2}$ hrs to 24 hrs.

Diarrhoea- Drugs causing any irritation or disturbance in GIT,it leads to diarrhea, there was no diarrhea observed in animals.

Mortality- sample 1(6-amino-3-hydroxy-4-(4'-methylphenyl)-2*H*chromen-2-on) was administered in the dose of 5,50mg/kg of body weight and no death had been occurred. But at the dose of 300mg/kg of body weight, in 24 hrs 2 mice were dead. So LD_{50} of sample 1 was fixed at 200mg/kg of body weight. Sample 2 (3, 7, 4', 5'-Tetrahydroxy-6- methoxyflavone) had been administered at a dose of 5,50mg/kg of body weight, no death was found. But at the dose of 300mg/kg of body weight, in 12 hrs no mice were found dead and at 24 hrs 2 mice were dead. So LD_{50} of sample 2 was fixed at 200mg/kg of body weight as per OECD Guidelines 423.

CONCLUSION

The present results shows isolated compounds from *Leptadenia reticulata* -sample 1 (6-amino-3-hydroxy-4-(4'-methylphenyl)-2*H*chromen-2-on) and *Lawsonia inermis* -sample 2(3, 7, 4', 5'-Tetrahydroxy-6- methoxyflavone*)* does not cause any harmful toxic effects and LD_{50} value which is calculated as 200mg/kg .In future a detailed pharmacological evaluation should be proceeded using this data.

REFERENCE

Bhatt T, Jain V, Jayathirtha MG, Banerjee G, Mishra SH. *In vitro* regeneration of roots of *Phyla nodiflora* and *Leptadenia reticulata*, and comparison of roots from cultured and natural plants for secondary metabolites (2006)," Indian J. Exp. Bio, 40:1382–1386.

Chauhan, N S, Saraf DK, Dixit VK. Effect of vajikaranrasayana herbs on pituitary gonodal axis (2010)," Eur. J . Integr. Med, 2: 89 – 91.

Chopra RN, Nayer SL, Chopra IC. Glossary of India Medicinal Plants, CSIR Publications, New Delhi (1956)," pp. 151.

Dandiya pc, chopra ym. Cns-active drugs from plants indigenous to india (1970)", Indian j.pharm, 2:67–90.

Frank A, Barile . Principles of Toxicology Testing (2008)," 1: 73-86.

Humber JM. The role of complementary and alternative medicine Accomodating pluralism.(2002)", J Am Med Assoc, 288:1655-1656.

Kalimuthu K. Prabakaran R. Preliminary phytochemical screening and GC - MS analysis of Methanol extract of ceropegia pusilla; (2013)", International Journal of Research in Applied, Natural and Social Sciences,1 (3): 49-58.

Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP, Chu I, Goddard M, Segal L, Springer JA, Myers RC. Comparison of the Up-and- Down, Conventional LD50 and Fixed Dose Acute Toxicity Procedures (1995)", Fd Chem Toxicol, 33: 223-231.

Newman DJ, Cragg GM, Snader KM. Natural Products as Sources of New Drugs (2003)", J Nat Prod, 66: 1022-1103.

Prosen H, Antonic J, Klobcar A, Determination of some organochlorine compounds in herbal colouring agent henna (*Lawsonia inermis*) and in tea (*Thea sinensis*), Arh Hig Rada Toksikol (2005)", 56, 1-7.

Rang HP, Dale M, Ritter (2001)", J. *Pharmacology*. Volume 13. 4th ed. New York, NY, USA; Churchill Livingstone.

Reddy KR. Folk medicine from Chittoor district Andhra Pradesh, India used in the treatment of jaundice (1988)", International Journal of Crude Drug Research, 26:137-140.

Shetty A, Deepa S, and Alwar MC, Acute toxicity studies and determination of median lethal dose,93(7), (2007)", 917.

Shivalinagappa. H., Satyanaryan. ND., and Purohit. MG.Antimplantation and pregnancy interruption efficacy of *Rivea hypocrateriformis* in the rat. (2001) ", J Ethnopharmacol, 74: 245-9

Tripathi KD. Essentials of Medical Pharmacology. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd: (2008):

Vaghasiya YK, Shukla VJ, Chanda SV. Acute oral toxicity study of Pluchea arguta boiss extract in mi ce. (2011)", J Pharmacol Toxicol, 6: 113-123.

Vaidya BG, Nighantu A, Shri Swami Atmanand Saraswati Ayurved Sahakari Pharmacy Ltd, Surat, (1965)", p.1106.