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Acute and Sub-chronic Oral Toxicity Studies of a Mineralo-herbal Drug Amlena on Experimental Rats

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Abstract: Amlena is a branded mineralo-herbal drug used to treat peptic ulcers. Purpose of our study was to explore the acute and sub-acute oral toxicities of drug Amlena in experimental rats. In acute toxicity studies the drug Amlena 5000 mg/kg was administered orally, observed after 1hr dosing and also observed for 14 days. In sub-chronic oral toxicity study, evaluations were carried out after administering daily oral doses of 500 and 1000 mg/kg body wt., for 28 days to the rats. Biochemical and haematological assessments as well as body and relative organ weights of the rats, gross necropsy was carried out. Results were statistically analysed using Analysis of variance (ANOVA) with the level of significance set at $P < 0.05$. The limit dose of 5000 mg/kg did not cause any mortality or signs of acute toxicity in the rats tested during the observation period. In the sub-chronic tests, the results did not show any significant variations in terms of haematological and biochemical parameters. The weekly body and organ weight of the rats showed no statistically significant differences between the control and treated rats.

Keywords: Amlena, Peptic ulcer, Acute oral toxicity, Sub-acute oral toxicity

INTRODUCTION

Amlena is mineralo-herbal drug, prescribed by prescribers for the treatment of peptic ulcer. Mineralo-herbal means the drug which contains both minerals as well as the herbals[1]. Peptic ulcer is one of the major gastro-intestinal disorders, which occur due to an imbalance between the aggressive (acid, pepsin, bile, alcohol, stress and NSAIDs) and defensive (mucus, bicarbonate, blood flow and restitution of epithelial) factors[2-3]. There are number of mineralo-herbal formulations consisting of minerals and herbal plants which are prescribed by the local prescribers but their scientific evaluation is not made yet. After realizing the potential use of these mineralo-herbal formulations in ulcer treatment, the present study was undertaken to evaluate acute and sub-chronic oral toxicities of drug Amlena.

MATERIALS AND METHODS

Mineralo-herbal formulation

Amlena, which is being used largely by the patients for its extremely good therapeutic value and its active constituents as Avipattikar Churna (200mg), Sitopaladi Churna (100mg), Swarn Makshik Bhasma (15mg), Giloy (15mg), Baratika Bhasma (2.5mg), Saunf (50mg), Anardana (10mg), Elaichi (10mg), Sona Geru Sudh (40mg), Sutshekhara (25mg), Amra (0.5mg), Gum (10mg). It is purchased from the local market of Jhansi, UP (India), and manufactured by Shree Dhar Ayurvedic Pharmacy, Jhanshi (UP) India.

Animals

Swiss albino rats of both sexes were taken from the animal house of Department. All animals were kept in cages in groups but the numbers of animals per cage were six so that there was no interference with clear observations of each animal, five days before experiments were started. Temperature of animal house was kept at 22 ± 3^0C, relative humidity at 65% and with the help of artificial light the 12 hrs light/ 12 hrs dark cycle was maintained. Beddings were changed three times per week. The animals were fed with standard diet and water *ad libitum*. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Institutional Animal Ethical Committee (IAEC) affiliated to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (CPCSEA- 837/ac/2004).

Acute oral toxicity

The highest attainable dose 5000 mg/kg was used in Organization for Economic Cooperation Development

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(OECD) guideline 423. Three female rats, each sequentially dosed at intervals of 48 hrs, were used for the test. Once daily cage side observations included changes in skin, fur, eyes, mucous membrane (nasal), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks[4].

Sub-chronic oral toxicity

Repeat-dose oral toxicity study was carried out according to OECD guideline 407. The animals were divided into three groups of 10 animals each (5 males and 5 females). Group 1 received 10 ml/kg body weight of distilled water and served as control. Groups 2 and 3 received doses of 500 and 1000 mg/kg body wt, respectively. The drug was administered daily for 28 days the same time daily and observed at least twice daily for morbidity and mortality. Body weights of the animals were evaluated weekly[5].

Haematological and biochemical analysis

During the sub-chronic oral toxicity, on the 29th day, after an overnight fast, the rats were anaesthetized with ether and blood sample for haematological and biochemical analysis were collected into tubes with and without EDTA, respectively. Haematological analysis was performed by assessing Haemoglobin (gm/dl), haematocrit (%), red blood cell count; RBC ($\times 10^6$/µl), white blood cell count; WBC ($\times 10^3$/µl), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and platelet count[6-7]. Biochemical analysis was performed on serum obtained after centrifugation of total blood (without anticoagulant) at 2500 rpm for 15 min. Standardized diagnostic kits were used for spectrophotometric determination of the following biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, alkaline phosphatase, glucose, total proteins and urea[8].

Gross necropsy

All animals in the study were subjected to a full, detailed gross necropsy like careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents were examined. Organ weights (heart, kidney, liver, spleen and testis) were also recorded.

Statistical analysis

All the *in vivo* experimental results were expressed as mean ± S.E.M. Data were analyzed by analysis of variance (ANOVA), with the level of significance set at $P < 0.05$.

RESULTS AND DISCUSSION

Acute oral toxicity

In Acute oral toxicity studies, it was found that the animals were safe up to a maximum dose of 5000 mg/kg body weight as per organization for economic co-operation development (OECD) guidelines which falls under class five values. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed.

Sub-chronic oral toxicity

In sub-chronic oral toxicity studies (the doses chosen on the basis of 1500-3000 mg of the drug per 60 kg body weight of human beings and which may be 10 to 20 times for the sub-chronic toxicity studies. In this regard, here, in our experiment for chronic toxicity studies the dose after calculation was taken as 500 mg/kg and 1000 mg/kg body weight of rats) it was found that there were no changes in normal behaviour pattern and no signs and symptoms of toxicity. Similarly, no significant differences in body weight
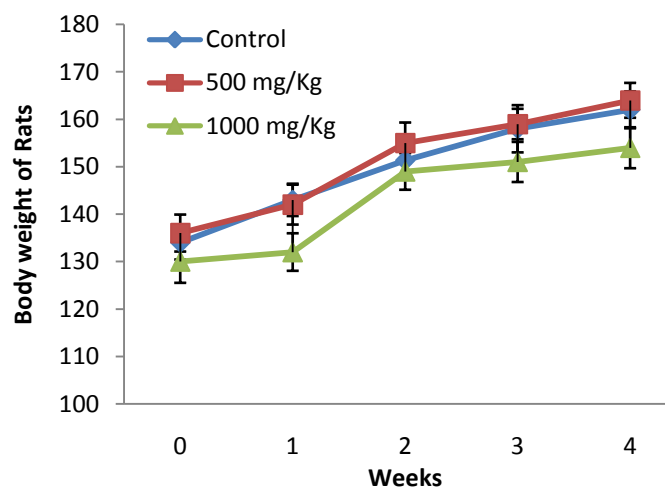

Fig 1. Results were expressed as mean body weight of rats after 28 days treatment with drug Amlena and are non-significant changes when compared with control rats (P> 0.05). All the values are Mean ± SEM; n= 10.

were observed between control and treated groups during this period (P > 0.05) and data is presented in figure 1.

There was small increase in the weight of liver in the treated group at dose level 1000 mg/kg but is not

statistically significant (P > 0.05). A slight decrease in the weight of testis in treated group was found in comparison to control group and may be considered as variations close to or within the normal range. Weights of other organs are not changed significantly and are presented in figure 2.
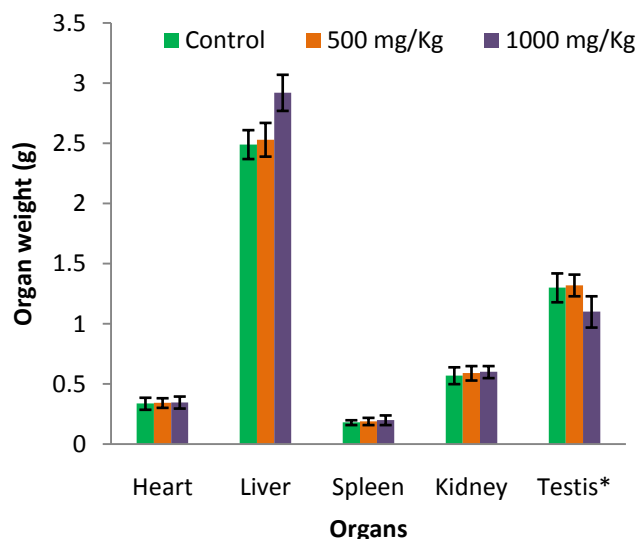

Fig 2. Results were expressed as mean ± SEM (n = 10) of rats after 28 days treatment with drug Amlena. AML treated groups showed non-significant changes as compared with control rats (P > 0.05).   * Means of 5 rats only.

Although a small decrease in RBC counts in comparison to control group were noted but was found within the limits. Other haematological parameters were also within the normal range and are shown in the table 1. Amlena is not showing the toxicity in the circulatory system even in the high dose.

The serum enzyme levels, total protein levels and creatinine remain close to the control values indicating that there were no deterioration in the function of liver and kidney which further suggests that on chronic administration of the drug amlena neither impaired the physiology of the kidneys and liver nor the cellular structures of the liver and kidneys[8]. The level of urea and blood sugar are statistically insignificant (P > 0.05). The biochemical parameters for rats after 28 days of treatment with two different doses are presented in the table 2.

So, in a conclusive way it may be said that the drug Amlena as found to be well-tolerated by rats. Acute oral toxicity studies in rats using the highest attainable dose of 5000 mg/kg for 14 days did not cause any lethal effect indicating that LD_{50} if any, should be higher than this dose. Organ weight and haematological parameters also remain close to or within the normal range suggesting no side effect. Twenty eight days toxicity studies with 500 mg/kg and 1000 mg/kg also revealed no lethal action and no adverse effect on the organs as indicated by no change of organ

Table 1. Haematological parameters for rats after 28 days treatment with drug Amlena at different doses

Parameters	Control	500mg/kg	1000mg/kg
Haemoglobin (g/dl)	12.30 ± 0.91	12.50 ± 0.88	12.62 ± 1.08
RBC (×10^6/μl)	8.46 ± 0.41	8.30 ± 0.56	8.37 ± 0.63
WBC (×10^3/μl)	7.31 ± 0.48	7.13 ± 0.27	7.12 ± 0.29
Haematocrit (%)	35.11 ± 2.21	35.65 ± 2.30	35.82 ± 2.38
MCV (fl)	43.00 ± 3.12	43.33 ± 3.40	44.50 ± 3.21
MCH (pg)	14.5 ± 0.42	15.10 ± 0.45	15.1 ± 0.32
MCHC (%)	35.03 ± 0.52	35.06 ± 0.56	35.23 ± 0.62
Platelet (×10^3/μl)	852 ± 34	932 ± 42	970 ± 54

Results were expressed as mean ± SEM (n = 10). Amlena treated groups showed non-significant changes as compared with control rats (P > 0.05).

Table 2. Biochemical parameters for rats after 28 days treatment with drug Amlena at different doses

Parameters	Control	500mg/kg	1000mg/kg
Urea (mg/dl)	16.47 ± 1.98	16.27 ± 1.24	16.07 ± 1.29
Sugar (mg/dl)	117.16 ± 3.28	117.5 ± 3.22	118 ± 4.42
Alkaline Phosphate (U/L)	260 ± 40	223 ± 24	232 ± 34
AST (U/L)	234 ± 12	209 ± 16	218 ± 23
ALT (U/L)	65 ± 5	59 ± 6	57 ± 4
Total Proteins (g/dl)	5.8 ± 1.2	5.9 ± 1.6	6.4 ± 1.5
Creatinine (mg/dl)	8.2 ± 0.5	7.8 ± 0.4	7.3 ± 0.5

Results were expressed as mean ± SEM (n = 10). Amlena treated groups showed non-significant changes as compared with control rats (P > 0.05).

weight. Haematological parameters and some specific serum enzyme levels remain also close to the control values.

Acknowledgement

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