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Acute toxicity evaluation of Elva in crude and processed forms in wistar rats

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Abstract

Background and objective: Herbal medicines are preferred mainly because of their safety, but some important drugs obtained from natural sources are highly toxic in nature whereas some are moderately harmful. However, toxic and harmful drugs are used after subjecting them to some processes of detoxification either to remove the element of harmful effects completely or at least to minimize it. *Elva, the test drug;* is said to be harmful for the various organs of the body, therefore, the drug are used after making it *Mudabbar* (Detoxified). The test drugs were named as EGM (*Elva ghair mudabbar*) and EM (Elva *mudabbar)* were subjected to acute toxicity study according to OECD Guidelines 423. The study was carried out in non-pregnant female Wistar rats of 150-200 gm. The animals were randomly selected and kept in standard laboratory conditions. The dosing for *Elva* was started from 2000 mg/kg dissolving in Carboxymethyl Cellulose (CMC) in three animals per group. The animals were randomly selected and kept in standard laboratory conditions. Features of acute toxicity included general observation, haematology, biochemistry and histopathology. No significant changes except increased HDL-C in EGM were recorded in EM. However, the harmful effects may be a precursor of toxicity if the drugs are used in overdose or for prolonged periods.

Keywords: unani medicine; *Mazarrat*; *Mudabbar*; toxicity; *Elva*; *Aloe barbedensis*

Introduction

Elva (*Aloe barbadensis* (L.) Burm. F.) commonly known as Aloe, is a plant in the Xanthorrhaceae family [1]. It is the oldest medicinal plants documented in the history. It is not only a medicinal substance, but has also been mentioned as a plant for beauty [2]. It is indigenous to Africa and Mediterranean countries. It grows wild in the islands of Cyprus, Canary cape, Malta, Sicily, Cape Verde and arid tracts of India [3]. There is many therapeutic actions of various parts of this plant has been stated such as abortifacient, antidiabetic, antimicrobial, antiviral, anti-inflammatory, anti-ulcer, anxiolytic, anti-oxidant, cardioprotective activity, haemodynamic, hypolipidimic, hepatoprotective, immunomodulatory, nephroprotective as well as wound healing [4].

In Unani traditional dried juice of Aloe leaf are used to treat *Bawaseer* (Haemorrhoids), *Inteshare Sha'ar* (Hair fall), *Deedane Ama* (Antihelminthic), *Dard-e-Ser* (Headache), *Ehtebase Tams* (Amenorrhoea), *Ezame Tihal* (Spleenomegaly), *Indemale Qurooh* (Wound Healing), *Iltehabe Meda* (Gastritis), *Yarqan,* (Jaundice), *Malankholia* (Malancholia), *Nawaseer* (Nasal polyps), *Nafsuddam* (Haemoptysis), *Qabz* (constipation), *Shiqaqe Miqad* (Fissure in Ano), *Waja-ul-Mafasil* (Arthritis), *Warme Kabid* (Hepatitis), *Zoaf-e-Meda* (Gastric weakness) [5, 6, 7].

The main chemical composition are investigated such as 7-Hydroxyaloin, Aloe-emodin, Aloesaponarin I&II, Aloin A and B (barbaloin), Anthranol, β-Carotene, apart from these compounds many amino acids, minerals sugars, organic acid are also found in this plant. Some new compound have isolated from this plant like Echitamine, Picrinine [8, 9]. In Unani system of medicine toxic and harmful drugs are used after subjecting them to some processes of detoxification either to remove the element of harmful effects completely or at least to minimize it. *Elva* is said to be harmful for the various organs of the body such as harmful for liver and stomach [10, 11].

Therefore, these drugs are used after making them *Mudabbar* (detoxified). It is said that *Elva* and such other drugs Loose their harmful effects after detoxification, but no data regarding the harmful effects in crude form and safety in processed form are available, therefore in the present study this drug is evaluated in crude and processed form on the parameters of acute toxicity to establish safety profile.

Materials and Methods

The present study entitled "Acute toxicity evaluation of *Elva* in crude and processed forms" was undertaken in the department of Ilmul Advia, National Institute of Unani Medicine, Bangalore. Before starting the experiment, the research protocol was submitted for ethical clearance. The institutional Animal Ethics Committee (IAEC) of the National Institute of Unani Medicine, Bangalore approved the protocol vid Reg. No. IAEC/IX/02/IA.

1. Preparation of test materials

Aloe dried juice was procured form local market Bangalore India, The test drug was identified and authenticated by Dr. Umesh Khumar Tiwari, Senior Research Associate, FRLHT, Bangalore,. The voucher specimen (FRLHT No. 3036) was deposited at herbarium of Institute of Ayurveda and Integral Medicine (I-AIM); other materials like apple

and wheat flour were also procured from the local market of Bangalore. The drug was processed before use by putting inside apple (*Malus domestica* Borkh.) fruit. Then dough of wheat was applied around the fruit and kept in fire till the dough becomes brown. The fruit was then removed from the fire and the drugs were taken out and dried at room temperature, and it was powdered finely in mortel and pestle and stored in air tight container. The powder was subsequently reconstituted in distilled water to the final concentration required for the concentration. 1 % carboxymethyl cellulose (CMC) in distilled water was served as control vehicle in the experiments.

2. Animals

A total no. of 24 female Wistar rats of 150-200 gm and 8-12 weeks old purchased from registered breeder. In acute oral toxicity study the animals were randomly selected and kept in their cages for at least 5 days prior to dosing to be allowed for acclimatization to the laboratory condition. Three animals in each group were kept under standard environmental condition, i.e. temperature 22± 3 ^{0}C and humidity 45-55 % with 12 hrs light and dark cycle. The animals were given standard pellet diet and tap water *ad libitum.* The animals care procedure and experimental protocols were in accord with the guidelines of CPCSEA. The animals were fasted for overnight, but water was withheld only for 3-4 hours and food further withheld 3-4 hours after dosing. Before starting the experiment, the research protocol was submitted for ethical clearance. The institutional Animal Ethics Committee (IAEC) of the National Institute of Unani Medicine, Bangalore approved the protocol vid Reg. No. IAEC/IX/02/IA**.**

3. Acute oral toxicity study

The two forms of drug were subjected to acute toxicity study, according to the Organization for Economic Cooperation and Development (OECD) Guideline for the Testing of Chemicals No. 423, adopted March, 1996 (OECD, 2001) [16]. The study was carried out in healthy, young adult, nulliparous and non-pregnant female Wistar rats of 150-200 gm and 8-12 weeks old. After acclimatization animals were randomly selected and kept 3 animals in each group for stepwise procedure. The animals were fasted for overnight, but water was withheld only for 3-4 hours. Next morning, the animals were weighed and the powdered material of test drugs in the form of suspension was administered in graded quantities by oral route. After administration of test drug, food was withheld for further 3-4 hours. The dosing was started 2000 mg/kg dissolving in Carboxymethyl Cellulose (CMC) in three animals per group.

All animals were observed individually for clinical signs of toxicity immediately after dosing and at 30 minute, 4 hrs and continued periodically during the first 24 hrs. Special attention was given during the first 4 hrs and daily thereafter, for a total of 14 days. Observations included evaluation of skin and fur, eyes, respiratory effects, autonomic effects such as salivation, diarrhea, urination and central nervous effects including tremors and convulsions, changes in activity, gait and posture, reactivity to handling or sensory stimuli and altered strength.

4. Food consumption

Food intake recorded on alternate day. A known amount of a diet was given to the animals daily. Early in the morning, the feed was reweighed and the amount consumed was calculated by difference.

5. Body weight

Body weight of the animals was taken weekly during the experimental
period.

6. Organ weight

Heart, liver and kidney were quickly removed, cleaned with saline, weighed and preserved in 10% formalin solution for histopathological analyses.

7. Hematology

At the end of the experiment, all the animals were sacrificed after 12 hrs fasting by the overdose of Theopentone sodium (50 mg/kg) and blood was withdrawn through heart puncture and collected in EDTA containing tubes for hematological examinations and in centrifuge tubes for biochemical examinations. R.B.C and W.B.C were counted by Improved Neubauer Haemocytometer and haemoglobin was estimated by haemoglobin reagent Cyanmethaemoglobin method by auto-analyser (Star 21 plus).

8. Biochemistry

Various biochemical tests *viz*. SGPT, SGOT, Serum Albumin, Total Protein, Blood Urea, Serum Creatinine, Uric Acid, Total cholesterol, Triglyceride and HDL-C of the blood sample were carried out by using standard kits and an auto analyzer (Star 21 Plus).

9. Histolopathological examination

After collection of blood, Liver, Kidney and heart were dissected out from the body and gross examination was done, the above mentioned three organs were weighed and preserved into 10% formalin buffer overnight, dehydrated and finally embedded in paraffin through histokinetic processing. Sections of 5-µm thickness were cut, stained with routine haematoxylin and eosin. Histopathological changes were examined under a bino-ocular microscope.

Analysis of Data

Statistical significance was determined by one-way analysis of variance (ANOVA) for food consumption, body weight, organ weight, haematological examinations and biochemical analyses. The data were expressed as Mean ± SEM and the values for the test and control groups were compared by using Kruskal-Wallis Test (Nonparametric ANOVA). The significance level was considered ($p< 0.05$.)

Results and Observations

Group of animals administered EGM and EM 2000 mg/kg and 5000mg/kg did not produce significant changes in behavior, skin effect, breathing defecation, postural abnormalities, impairment in food intake and water consumption and yellowing or loss of hair. No mortality of animal was observed during the experimental period.

1. General Observations

According to the Paragraph 24 and 25 of the OECD Guidelines (423), Wellness parameters of animals were observed continuously during the first 30 min after dosing

and observed periodically with special attention given during the first 4 hours and for the next 24 hours and then daily thereafter, for 14 days. Observations included changes in skin and fur, eyes and mucous membranes and behavioral pattern. All observations were recorded systematically.

2. Effect of EGM and EM on body weight

The animals of all groups showed a slight weight gain throughout the experiment but statistically not significant.

3. Effects of EGM and EM on food intake

Food intake was measured alternate day of all animals for 14 days. Mean of food intake of EGM and EM at the dose of 5000 mg/kg was found to be 55.14±6.727, 86.14±6.544 and 74.29±5.172 gm. No significant difference was found respect to control except in Control vs EGM, it was found statistically significant (P<0.0159)

4. Effect of EGM and EM on liver, heart and kidneys weight

Table no. represented no statistical significant differences in the weight of each organ between test and control group. Except the Heart weight significantly increased in EGM only in reference to Controls.

5. Haematological analysis

Haemoglobin, RBC and WBC count in Controls, EGM and EM were found statistically not significant as compared to Controls.

6. Biochemistry

A little variation in was observed between control and treated groups but it was not statistically significant, indicated the healthy status of liver, kidney and heart except total cholesterol level was found significantly increased in EGM (P<0.05) as compared to Controls (2).

Table 1: Biochemical analysis of EGM and EM and control groups of animals.

Parameters	Control	EGM	EM
SGPT	49.7933±11.3913	40.6767±4.6287	57.4033±4.7048
SGOT	148.0967±26.8049	117.3±8.0525	110.8333±3.71
Total Protein	2.806±0.4809	3.4593±0.1397	2.796±0.3795
S. Albumin	3.5157±0.683	3.1763±0.2188	5.375±0.1524
S. Urea (mg/dl)	28.5167±5.7717	21.69±2.763	25.7667±1.6058
S. Creatinine (mg/dl)	0.7067±0.0761	0.594±0.1127	0.9233±0.3876
S. Uric Acid (mg/dl)	2.73±0.8018	1.5433±0.0471	1.4397±0.2306
Triglyceride (mg/dl)	108.55±43.761	90.7633±4.7621	282.8667±79.6771
Total Cholesterol (mg/dl)	50.3467±4.5041	47.0467±8.6361	90.9033±11.6189
HDL-C (mg/dl)	29.7867±3.7312	102.9867±32.2451*	56.5633±2.8922

7 Histopathological Examinations

In the Control group, section studied from the myocardium shows the intact arrangement of the cardiac muscle fibers. The cardiac muscle fibers showed intact integrity of myocardial cell membrane, myofibrillar structure with striations and continuity with adjacent myofibrils. The interstitial space appeared unremarkable. The vascular spaces appeared unremarkable.

In EGM, section studied from the myocardium showed the intact arrangement of the cardiac muscle fibers. Some of the cardiac muscle fibers showed loss of integrity of myocardial cell membrane, myofibrillar structure with lack of striations and loss of continuity with adjacent myofibrils. The interstitial space appeared unremarkable. The vascular spaces appeared unremarkable.

In EM, section studied from the myocardium showed intact arrangement of the cardiac muscle fibers. Most of the cardiac muscle fibers showed loss of integrity of myocardial cell membrane, myofibrillar structure with lack of striations and loss of continuity with adjacent myofibrils. The interstitial space in some areas appeared to increase with mild edema. The vascular spaces appear unremarkable.

In Control group, section studied from the liver parenchyma showed intact architecture. The hepatocytes in the perivenular region. periportal region and midzonal region appeared unremarkable. The hepatic parenchyma, central veins and sinusoids appeared unremarkable.

In EGM, section studied from the liver parenchyma showed intact architecture. The hepatocytes in the perivenular region, periportal region and midzonal region appear unremarkable. Focal inflammatory infiltration was seen in the periportal region. The hepatic parenchyma and central veins appeared unremarkable. The sinusoids appear dilated.

In EM, section studied from the liver parenchyma shows intact architecture. The hepatocytes in the perivenular region, periportal region and midzonal region appeared unremarkable. Focal inflammatory infiltration was seen in the periportal region. The hepatic parenchyma and central veins appeared unremarkable. The sinusoids appeared dilated and congested.

Discussion

Unani physicians have devised various methods of detoxification depending upon the nature and use of a drug. The processing methods are collectively known as *Tadbeere Advia*. Some methods like Tashwiyah, *Tahmees, Ghasl, Irgha, Tadheen, Taklees, Ihraq, Sokhta and Nakhl, Tasweel* etc. are some of important methods. All the methods are not aimed always at removing the harmful parts of the drugs. In certain cases like *Irgha,* is used just to remove the impurity of drugs, *Ghasl* is also meant for the same purpose.[12] *Tashwiyah* is a process in which the drug which is to be processed is put in fruits like quince or apple by making a cavity in the fruit then the removed part of the fruit is kept over it and the fruit is then wrapped in cloth over which kneaded flour is applied; this preparation is then dried and subjected to fire of low intensity usually made of ignited dung cakes or saw dust (here saw dust was used for the purpose) till the preparation becomes brown. *Elva* is usually processed by this method. *Elva* were processed by *Tashwiyah* [13]. *Elva* is used after detoxification, but no data are available on adverse effects due to use of drugs without processing and safety after processing. It is also not very clear that what changes occur in the drug after processing.

All these assertions are based on observations of Unani physicians. Keeping these points in mind *Elva* were selected for acute toxicity studies for generating data on the use of these drugs before and after processing of the same. Acute toxicity studies in animals are normally necessary for any new pharmaceutical proposed for human utilization or when a dosage form is changed. The data acquired from these studies are useful in the identification of target organs of toxicity [14]. Acute toxicity is a single dose of chemical substance, biological toxin or radionuclide that causes such severe organ damage or disruption of body function [15]. For acute toxicity study of *Elva* is pre and post processing, OECD guidelines (423) was followed [16]. Parameters such as general observations haematology, biochemistry and histopathological examinations were considered. In general observation, body wt., food intake, diarrhea, skin pigmentation and colour, palpable mass, motility convulsion and tremor were observed. In haematology effect of drug on RBC, TLC and Haemoglobin, whereas in biochemistry estimation of SGOT, SGPT, Total Protein, Albumin, Serum Urea, S. creatinine, S. Uric acid, T. Cholesterol, and HDL-C were considered as parameters for toxicity. In histopathology liver, kidney and heart were examined microscopically for any damage.Since no such study has been carried out earlier, therefore no data are available to compare our results except some findings on acute toxicity of *Elva* and that too without processing. Therefore, our findings may be considered as primary findings.

In our study no significant change in body weight in case of both drugs neither in crude form, nor after in processed form was observed in animals throughout the study. In a study conducted by Saritha *et al* [17], similar results have been shown for *Elva*, which was carried out on the sample used without processing. When the effect of EGM and EM was observed in liver, heart and kidney weight, it was found that only the heart weight increased significantly in EGM. (0.7158 ± 0.014, $P<0.05$) with respect to Controls. No change was observed in weight of liver and kidney. In a study conducted by V. Saritha *et al* similar result has been shown for *Elva*, which was carried out on the sample used without processing. These results show that both *Elva* have no effect on body as well as organ weight. The significant difference in organ weights between treated and untreated (control) animals may also occur in the absence of any morphological changes [17, 18]. Changes in skin, increased motility, convulsion etc are important general observations usually found in toxicity of drugs. No findings have been reported regarding the effect of EGM and EM on skin colour, skin and fur, eyes, mucous membrane and behavioral pattern, motility, sleep, coma, tremors, convulsion, morbidity and motility. In our study too no such findings were observed.

Haematology is good parameter for assessment of toxicity. In our study, we assessed RBC count, Hemoglobin and TLC. When compared these parameters with that of Controls, no significant change was observed in any group. Hence, these parameters were of little importance regarding acute toxicity of *Elva* in both unprocessed and processed forms also, no data are available in this regard.

Liver and kidney damage are common manifestation of toxicity of many drugs. Therefore, liver function tests and renal function tests may be a reliable determinant of hepatic and renal injury. In our study SGOT, SGPT, Total Protein and Albumin were estimated to observe any remarkable change in liver indicating parenchymal injury. But as far as these parameters are concerned, no remarkable change was found with reference to these parameters. Similarly Serum Urea, Serum Uric acid, etc. are good indicators of renal function. These were estimated in our study to see any remarkable change in renal parenchyma but no significant change was found when compared with the Controls. As these parameters were normal in both crude and processed form of *Elva*. Hence it may be concluded that these drugs are relatively safe for liver and kidney. No data are available in this regard. Triglyceride, Total Cholesterol and HDL-C, etc. are also indicative of disturbance of metabolism of lipid which is found in toxicity. Regarding these parameters, only HDL-C was increased significantly ($P<0.05$) in EGM, but this parameter was not conclusive.

Since no remarkable toxicity was observed, which is evident from most of the parameters applied in this study, it may be concluded with some degree of certainty that the term *Mazarrat* (harmful effect) is not the appropriate term for toxicity. In conventional medicine too harmful/adverse effects have been mentioned as two different conditions with regard to adverse effects of drugs. The adverse effect is a broader term which includes any undesirable or unintended consequence of drug action, whereas toxicity (toxic effects are the result of certain drugs occurring due to overdoses or prolonged use generally observed in liver, kidney, brain and heart [19, 20].

Since the study was designed as an acute toxicity study for evaluation of the toxic (harmful) effect of *Elva* and the toxic effects were mainly observed in relation to liver and kidney. But in Unani medicine the drug has been mentioned to be harmful for liver, kidney and heart. Therefore, harmful effects (*Mazarrat*) and toxicity should not be mixed. However, it may be concluded that harmful effects are precursor of toxicity if a drug is used in overdose or for prolonged periods. Since this study was of short duration therefore it is quite possible that in spite of being toxic in nature, these drugs have not exerted toxicity due to use for a very short period. However the study showed moderate signs of toxicity and the drugs seems to be relatively safe, but this study does not promise total safety of the drug. Detail toxicity studies are required in this regard.

Conclusion

Elva is used in processed form, however sometimes *Elva* is used internally without processing. Some studies on this drug in respect to pharmacological actions and standardization have been carried out, but as far as the toxicity study is concerned the data are scare. Therefore, in the present study this drug were selected for acute toxicity in order to generate data for establishing safety range of Elva and to see if there is any difference between the toxicity/adverse effect elva in crude as well as processed form. The data obtained by this study have been analyzed by appropriate statistical tests.

The study was designed as per OECD guidelines (423). The parameters include general observation (skin and fur, eyes, mucous membrane, salivation, lethargy, sleep, coma, convulsion, tremors, morbidity, body weight, organ weight, food intake, skin changes, palpable mass and mortality); haematology (RBC count, TLC and Haemoglobin estimation); LFT (SGOT, SGPT, T. Protein and Albumin); RFT (Uric Acid, S. Creatinine and S. Urea); Triglyceride, Total Cholesterol and HDL-C; histopathology of Heart, Liver and Kidney. The findings and conclusion are given

below: The effect of EGM and EM on body weight was not significant statistically however the weight increased slightly, The weight of Liver, heart and kidney increased slightly in EGM, EM, but significant increase in weight of heart was found only in EGM. RBC, TLC and Hb increased very slightly when compared with Controls but this increased overall not significant, SGOT and SGPT slightly increased in EGM and but not in EM. However, it was not significant statistically, but indicates mild toxic effects (adverse effect), Serum Urea was found to be increased slightly in EM. This finding was not conclusive, Serum creatinine was normal, Triglyceride slightly increased in SGM indicating that it may disturb lipid metabolism, HDL-C increased significantly in EGM showing that it may disturb lipid metabolism and Histopathological findings showed that both forms did not damage organs remarkably which is evident from the absence of noticeable necrosis.

No remarkable toxic signs and symptoms were observed with respect to parameters usually applied in acute toxicity studies, it may be concluded that *Elva* are harmful for the body in crude form which is evident from some findings. This has also been mentioned in Unani books. However in Unani books there is no clear cut description about *Mazarrat* (adverse effects) therefore it cannot be concluded that this drug is totally safe for organs as well as whole body. No significant difference was observed in crude and processed forms of research drugs except in respect to few parameters. So it may be concluded that there is not much difference between the two forms. However this drug should be used after processing suggested by Unani physicians unless it is evident from advance studies that there is no difference between the two forms. Further study, in this regard is required by a design that is based on the Unani concept of *Mazarrat* to assess the harmful effects of elva and other *Muzir* drugs. It may also be noted that harmful effects *(Mazarrat)* and toxicity should not be mixed.

Table 1: Observations of EGM treated group

Observations	30 Min		4 Hrs.		24 Hrs.		48 Hrs.		1 Week		2 Weeks	
	C	SGM	C	SGM	C	SGM	C	SGM	C	SGM	C	SGM
Skin and Fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Mucous Membrane	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Coma	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Convolsion	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Diarrhoea	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Morbidity	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

C=Control, EGM=*Elva* Ghair Mudabbar, N=Normal

Table 2: Observations of EM treated group

Observations	30 Min		4 Hrs.		24 Hrs.		48 Hrs.		1 Week		2 Weeks	
	C	SGM	C	SGM	C	SGM	C	SGM	C	SGM	C	SGM
Skin and Fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Mucous Membrane	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Coma	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Convolsion	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Diarrhoea	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Morbidity	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 3: Effect of EGM and EM on Body Weight (gms±SEM)

Groups	0day	8th day	15th day
Control	196±19.85	196±18.00	191.33±18.31
EGM	263.33±20.16	244.66±21.48	243.66±24.44
EM	244.33±12.33	253±14.50	249.66±14.85

N=3, Test used: Kruskal-Wallis Test (Nonparametric ANOVA). The P value is >0.05, considered not significant.

Table 4: Effect of EGM and EM on Food Intake (gms±SEM)

Control	EGM	EM
55.14 ± 6.727	86.14 ± 6.544*	74.29 ± 5.172,

Test used: Kruskal-Wallis Test (Nonparametric ANOVA), *-P<0.0159 with respect to Control. Considered significant.

Table 5: Effect of EGM and EM on Heart, Liver and Kidneys (g±SEM)

Groups	Heart	Rt. Kidney	Lt. Kidney	Liver
Control	0.6341±0.0191	0.6181±0.0539	0.6304±0.0314	5.499±0.5523
EGM	0.7158±0.014*	0.7104±0.0235	0.6925±0.0234	6.798±0.459
EM	0.6341±0.0177	0.6505±0.0118	0.6304±0.0161	5.899±0.2722

Test used: Kruskal-Wallis Test (Nonparametric ANOVA), *-P<0.05 with respect to Control.

Table 6: Effect of SGM and SM on Heart, Liver and Kidneys (gms±SEM)

Groups	Heart	Rt. Kidney	Lt. Kidney	Liver
Control	0.4997 ± 0.02147	0.6699 ± 0.03388	0.5972 ± 0.02152	6.643 ± 0.5280
SGM	0.5754 ± 0.09661	0.5910 ± 0.02955	0.6059 ± 0.02016	6.703 ± 0.7860
SM	0.4919 ± 0.01254	0.5767 ± 0.04396	0.5375 ± 0.03533	6.503 ± 0.6638

Test used: Kruskal-Wallis Test (Nonparametric ANOVA). The P value is >0.05, which is statistically not significant.

Table 7: Effect of EGM and EM on Haemoglobin, RBC and TLC

Groups	HB	RBC Count	TLC
Control	14.98333±0.6833578	4.6933±0.3497	11533.3333±196.4971
EGM	14.25±1.010413	5.3533±0.5904	9283.3333±467.5587
EM	14.49±0.8400595	4.6±0.4072	9200±841.1302

Test used: Kruskal-Wallis Test (Nonparametric ANOVA). The P value is >0.05, which is statistically not significant.

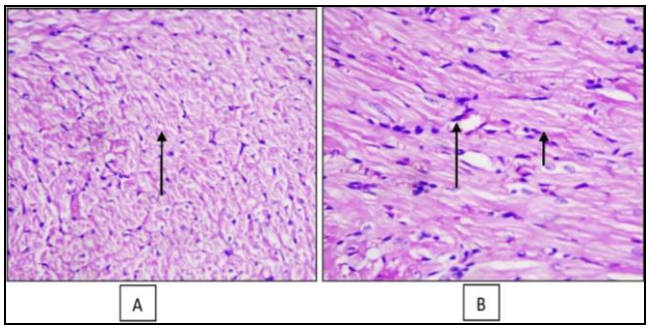

Fig 1: Histopathology of heart of control group *(Elva)*

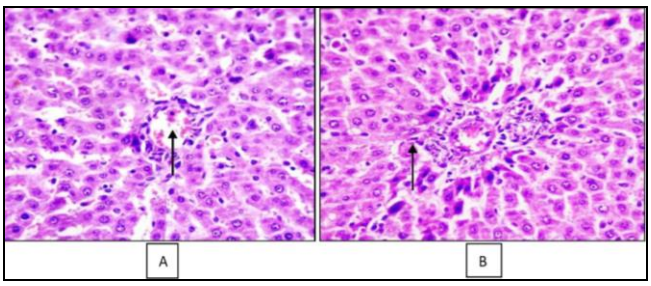

Fig 2: Histopathology of Liver of Control group *(Elva*)

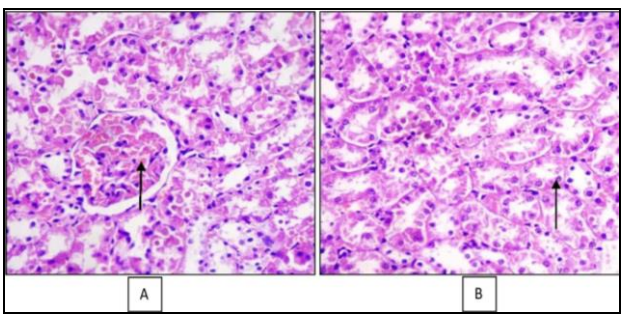

Fig 3: Histopathology of Kidney of Control group *(Elva*)

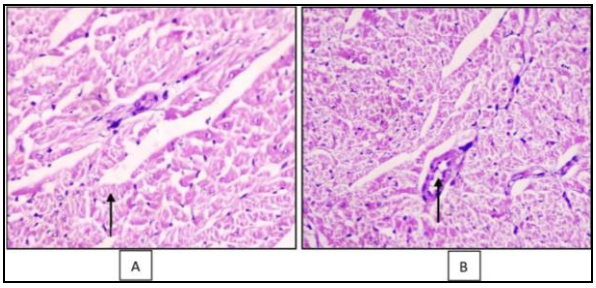

Fig 4: Histopathology of Heart of EGM group

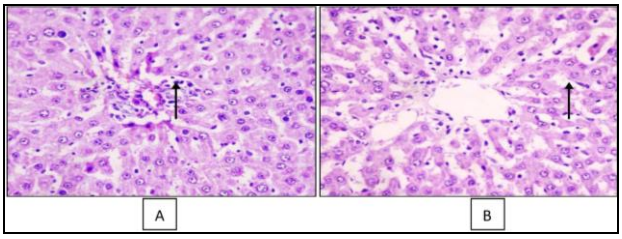

Fig 5: Histopathology of Liver of EGM group

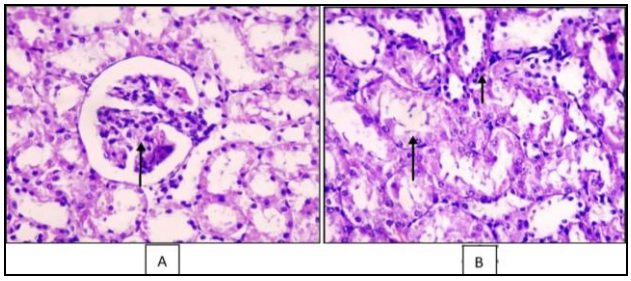

Fig 6: Histopathology of Kidney of EGM group

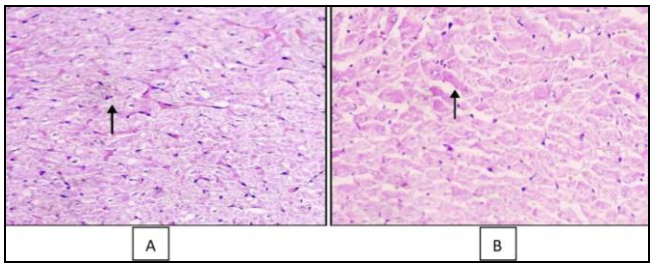

Fig 7: Histopathology of Heart of EM group

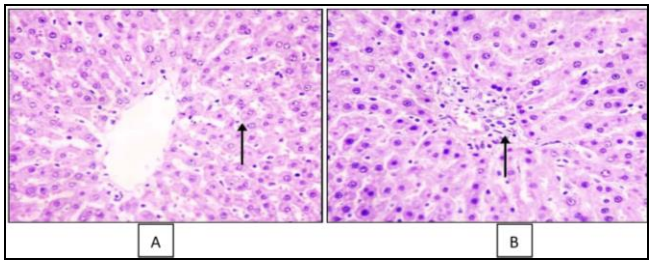

Fig 8: Histopathology of Liver of EM

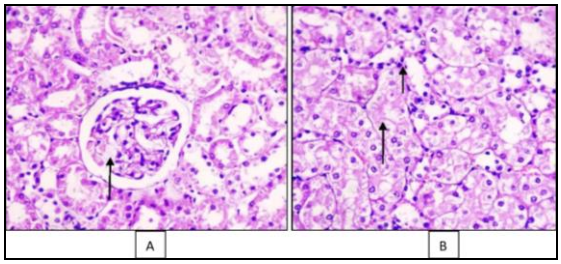

Fig 9: Histopathology of Kidney of EM group

References

1. Surjushe A, Vasani R, Saple DG. Aloe vera: a short review. Indian J Dermatol,2008:53(4):163-6.
2. Ahlawat KS, Khatkar BS. Processing, food applications and safety of aloe vera products: a review. J Food Sci Technol,2011:48(5):525-33.
3. Moriyama M, Moriyama H, Uda J, Kubo H, Nakajima Y, Goto A. Beneficial Effects of the Genus Aloe on Wound Healing, Cell Proliferation, and Differentiation of Epidermal Keratinocytes. PLoS One,2016:13:11(10):e0164799.
4. Gupta VK, Malhotra S. Pharmacological attribute of Aloe vera: Revalidation through experimental and clinical studies. Ayu,2012:33(2):193-6.
5. Ghani MN. Khazayinul Advia New Delhi: Idara Kitab ul Shifa, 308-312, 814-815.
6. Kabeeruddin H, Makhzanul Mufradat (Kitab al Advia). 2nd ed. New Delhi: Idarae Kitab ul Shifa, 2007, 259-260.
7. Hakim AH. Bustanul Mufradat Jadeed Musawwar. 1st ed. New Delhi: Idarae Kitab ul Shifa, 2002, 340-341.
8. Zhong J, Huang Y, Ding W, Wu X, Wan J, Luo H. Chemical constituents of Aloe barbadensis Miller and their inhibitory effects on phosphodiesterase-4D. Fitoterapia,2013:91:159-165.
9. Nadkarni KM. Indian Materia Medica. 3rd ed. Mumbai: Popular Prakashan Private Limited,2010, 73-74, 376.
10. Ibn Baitar ZbA. Al-Jami Li-Mufradat al Advia wa al Aghzia New Delhi: CCRUM, 1999, 51-54, 170-175.
11. Khafir HA. Majmaul Bahrain Lahore: Matba Munshi Naval Kishor, 157.
12. Zillurrahman H. Jadeed Unani Dawa Sazi: Idara Kitabul Shifa, 2002, 36.
13. Hakeem MK. Byaze Kabeer New Delhi: Idara Kitab al-Shifa, 2010, 10, 16, 40, 50, 53, 58, 154, 154.
14. Amgad A, Awad EG, Ismail MM, Abelkareem M, Elnazeer IH. Evaluation of Acute and Sub Chronic toxicities of the topical extract of Mangifera indica seeds on Wistar albino rats. International Journal of Research in Phytochemistry and Pharmacology,2013:3(3):149-154
15. Taboureau O. Otab@cbs.dtu.dk. [Online]. [cited] Feberaury 14. Available from: otab@cbs.dtu.dk, 2014.
16. OECD Guideline for Testing Chemicals 423-Acute Oral Toxicity – Acute Toxic Class Method. Guidelines. Paris, France: The Organization for Economic Co-operation and Development (OECD), 2001, 1-14.
17. Saritha V, Anilakumar KR. Toxicological evaluation of methanol extract of Aloe vera in rats. International Journal on Pharmaceutical and Biomedical Research,2010:1(5):142-149
18. Sina I. Al-Qanoon fil-tib Lahore: Matbo'aate Sulemani,1998:163(164):188-189.
19. Bennett PN, Brown MJ. Clinical Pharmacology. 9th ed. Delhi: Elsevier, a division of Reed Elsevier, India private Limited, 2005.
20. Guo X, Mei N, Aloe vera. A review of toxicity and adverse clinical effects. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev,2016:2:34(2):77-96.