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Acute Toxicity and *In-Vivo* Laxative Studies of the Triphala Extract in Experimental Animals

Revathi S*[1], Gopal V[2], Jeyabalan G[1]

[1] Sunrise University, Alwar, Rajasthan, India

[2] College of Pharmacy, Mother Theresa Postgraduate and Research Institute of Health Sciences, Puducherry, India

ABSTRACT

Triphala has been extensively used in traditional medicine for laxative, antidiabetic, expectorant, astringent, anti-aging etc. The acute toxicity of methanolic extracts of Triphala in 300 mg, 600 mg, and 1000 mg/kg has not yet been studied. The current studies were done by employing Swiss Albino mice as experimental animal. The methanolic extracts of Triphala were considered safe up to a dose of 1000 mg/kg when evaluated for acute oral toxicity in accordance with the OECD (Organization for Economic Cooperation and Development) guidelines. The results of acute toxicity showed no signs of toxicity such as general behaviour changes, mortality, changes on gross appearance or histopathological changes of the internal organs of rats. The examinations of signs of acute toxicity showed no abnormalities in the test groups as compared to the controls. Haematological and blood chemical values in treated groups were normal in comparison with the control group. Therefore, the extract of *Triphala* given orally to mice did not produce acute toxicities. The laxative activity on Albino Wistar rats shows that the Triphala extract has significant positive effect on constipated animals.

Keywords: Acute toxicity, Triphala, laxative, *in-vivo*

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***Address for Correspondence:**

Revathi S, Sunrise University, Alwar, Rajasthan, India

INTRODUCTION

The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world. Medicinal plants are being looked up once again for the treatment of diabetes. Many conventional drugs have been derived from prototypic molecules in medicinal plants. There are many herbal remedies suggested for diabetes and diabetic complications. Medicinal plants form the main ingredients of these formulations. "Triphala" was found to have the laxative, antibacterial antidiabetic and other beneficial effects in traditional medicine as listed in the table below.[1]

Triphala has been used in traditional medicine in India for over 1000 years according to the writings of the great physician Charak in a foundational text of Ayurveda called the Charaka Samhita as well as in another key text called the Sushruta Samhita. As both Ayurveda and Western medicine agree that health and disease begin in the gut, Triphala represents an essential foundational formula as it promotes efficient digestion, absorption, elimination, and rejuvenation. Numerous references in well-respected Ayurvedic medical texts make clear that Triphala is revered as a multiuse therapeutic and perhaps even panacea historically.[2]

Triphala is a botanical preparation comprised of equal parts of three herbal fruits: *Terminalia chebula, Emblica officinalis,* and *Terminalia bellirica*. Researchers found that *Terminalia chebula* increased gastric emptying by 86 percent. Recently published studies report that *Terminalia* exhibits antibacterial activity against a number of bacterial species. *Emblica officinalis* fruit is the world's richest source of natural vitamin C. Researchers have attributed many of traditional benefits of *Emblica officinalis* to its antioxidant properties.[3]

MATERIALS AND METHODS

Collection of fruits

The fruit specimens of *Terminalia chebula*, *Terminalia bellirica and Emblica officinalis* were collected in and around Rajahmundry, Andhra Pradesh. The fruit specimens were identified and authenticated by Dr. J. Suneetha M.Sc., M.Phil., PhD, Professor and Head, Department of Botany, Government Art College, Rajahmundry, Andhra Pradesh.

Specimen No: GAC/RJ/BO/2019/03/34. A voucher specimen was stored in our laboratory.

Preparation of Triphala

All the fruits of Triphala were cleaned to remove the foreign materials and dust. The seeds are removed and the pericarp and mesocarp of fruits were dried in shade. The dried fruits were then pulverised into fine powder using a stainless steel electrical mixer, passed through # 100 mesh sieves. Then the powders of *Terminalia bellirica*, *Emblica officinalis*, and *Terminalia chebula* are mixed in ratio of 1:2:4 and stored in an airtight container for extraction.

Fig 1: Dried *Emblica officinalis*

Fig 2: Dried *Terminalia chebula*

Fig 3: Dried *Terminalia bellirica*

Preparation of extract

An accurately weighed 50 gm of powder was subjected to hot percolation in a Soxhlet extractor (Sisco) with 500 ml of 75% of methanol at 80°C as solvent to get the methanolic extract. The Triphala powder is added in boiling methanol and macerated in a percolator for 2 h. Then the process of hot continous percolation was continued by adding methanol till the menstruum colour evanesces indicating the completion of the process. The resultant extract was concentrated using rotary vacuum evaporator (Buchi type – Sisco) below 40°C. This concentrated extract is evaporated to dryness over a water bath. Then the extract is stored in desiccator using silica gel.[4, 5]

Experimental animals[6]

Albino Swiss mice of 10 weeks old weighing 20 - 25 gm were obtained. Animals were maintained in a room with controlled temperature of (22 ± 3°C) for 12 hour (hr) light / 12 hour dark cycle with free access to food and water. Animal care and research protocols were based on the principles and guidelines adopted by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The acute toxicity study was carried out in both the animals' male as well as female Albino Swiss mice as per Organization Economic Cooperation Development (OECD) 423 guidelines. The experimental protocol was approved by the institutional Animal Ethics Committee GSP/IAEC/2019/02/03 following CPCSEA guidelines. Prior to dosing, animals were fasted 18 hours with water *ad libitum* before being weighed.

Evaluation of acute toxicity[6]

Prior to dosing, animals were fasted 18 hours with water *ad libitum* before being weighed and the dose was administered orally by gavage method. For the evaluation of acute toxicity, the dose levels of the methanolic extracts of 300 mg, 600 mg, and 1000 mg/kg body weight of mice was used. A separate set of control mice were given the vehicle only (distilled water). The animals were divided into four groups with six animals in each group. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter for about total of 14 days. At the end of 14 day's observation period, the animals were anaesthetized, and their blood samples were collected through cardiac puncture with and without anticoagulant (EDTA) for haematological and biochemical studies, respectively. For histopathological studies the liver and kidney of animals were excised and examined macroscopically and microscopically. These organs were then preserved in 10% v/v formalin for histopathological examinations by standard techniques.

Group 1: Normal control

Group 2: Minimum dose

Group 3: Medium dose

Group 4: Maximum dose

Gross behaviour study[7]

The animals were continuously monitored for every 30 min for first 4 hours (hr), periodically until 24 hrs for gross behavioural changes and mortality. Cage side observations include changes in skin & fur, eyes & mucous membrane, respiratory, circulatory, autonomic nervous system (salivation, lacrimation and defecation), somatomotor activity, central nervous system (ataxia, tremors and coma) and behavioural pattern changes were monitored once daily. Mortality, if any observed over a period of 14 days was also recorded.

Body weight analysis[8]

Changes in the body weight of the treated group animals were compared with the control group animals. Individual body weight of animal was recorded before the administration of a drug on 0 day of the study and on the 14th day of the experiment before withdrawal of the blood for all the animals.

Haematological study[6]

Blood samples were collected by retro orbital puncture of all the treated animals and control animals. Samples were analyzed for routine haematological parameters like hemoglobin, white blood corpuscles, red blood corpuscles, packed cell volume, hematocrit and platelet counts. Blood cell counts were done with blood smears. The above mentioned haematological parameters were analyzed.

Biochemical study[9]

For the determination of Serum Glutamic Oxaloacetate Transminase, Serum Glutamic Pyruvate Transaminase, albumin, triglycerides, sugar, protein and creatinine, blood samples were collected separately for each of the group (control and treated). The collected samples were centrifuged for 10 min at 5000 rpm/min and then analyzed.

Gross necropsy and histopathological studies[10]

After administration of drug at the end of 14th day, the animals were sacrificed by over dosage of anaesthetic ether – chemical method. All animals in the study were subjected to a full detailed gross necropsy like careful examination of the external surface of the body and abdominal cavities and their contents are examined. The organs like liver and kidney were excised, washed with cold saline. All the organ samples were fixed in buffered 10% v/v formalin and then sectioned and stained with hematoxylin and eosin stain for histopathological examination under light microscopy.

Determination of *in-vivo* laxative activity of the Triphala extract[11, 12]

Albino Wistar rats of both genders which are 8 – 16 weeks old weighing 150 – 200 g were used. All the animals were housed in artificial lighting sequence for 12 hours of dark & 12 hours of light cycle and at standard environmental conditions of room temperature 22 ± 3°C. In routine, all animals were acclimatized to the laboratory conditions for 5 days by giving standard diet and water *ad libitum*. Later on, the animals were divided into five groups with six animals in each group. All the animals were kept on fasting for 12 hr. Then, all the animals have received the doses respectively as mentioned below by gavage method. After 1 hour, the animals also received Loperamide (5mg/kg) except the group 1. The faeces production in all groups are monitored, collected and weighed for the next 24 hours. Animal care and research protocols were based on the principles and guidelines adopted by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (GSP/IAEC/2019/02/03).

Group 1: Normal Control.

Group 2: Negative Control

Group 3: Positive control

Group 4: 50 mg/kg of Triphala extract

Group 5: 100 mg/kg of Triphala extract

The dry weight determined after the faeces were dried for 8 hours at 70°C. The water content of the faeces was calculated as follows,

$$\%\ water\ content\ of\ faeces = \frac{wet\ weight\ of\ faeces - dry\ weight\ of\ the\ faeces}{wet\ weight\ of\ the\ faeces} * 100$$

RESULTS & DISCUSSION

Gross behaviour study

Gross behaviour study was carried out and there is no negative observation or adverse changes found and no odd behavioural changes or toxic signs in cage side observation of animals and the results are shown in the tables 1, 2 & 3. All the animals were found alive, healthy as well as active. There was no mortality observed in the experimental animals during the study period of 14 days as showed in the table 4.

Table 1: Gross behaviour study of treated animals (Group 1)

Observation	0.5 hr	1 hr	1.5 hr	2 hr	2.5 hr	3 hr	4 hr	1st day	14th day
Respiration	+	+	+	+	+	+	+	+	+
Convulsion	_	_	_	_	_	_	_	_	_
Writhing	_	_	_	_	_	_	_	_	_
Skin & fur	+	+	+	+	+	+	+	+	+
Touch sense	+	+	+	+	+	+	+	+	+
Ear	+	+	+	+	+	+	+	+	+
Eyes	+	+	+	+	+	+	+	+	+
Salivation	+	+	+	+	+	+	+	+	+
Urination	+	+	+	+	+	+	+	+	+
Diarrhoea	_	_	_	_	_	_	_	_	_
Lacrimation	_	_	_	_	_	_	_	_	_
Ataxia	_	_	_	_	_	_	_	_	_
Tremors	_	_	_	_	_	_	_	_	_
Coma	_	_	_	_	_	_	_	_	_
Mortality	_	_	_	_	_	_	_	_	_

*+ Normal – Absent

Table 2: Gross behaviour study of treated animals (Group 2)

Observation	0.5 hr	1 hr	1.5 hr	2 hr	2.5 hr	3 hr	4 hr	1st day	14th day
Respiration	+	+	+	+	+	+	+	+	+
Convulsion	_	_	_	_	_	_	_	_	_
Writhing	_	_	_	_	_	_	_	_	_
Skin & fur	+	+	+	+	+	+	+	+	+
Touch sense	+	+	+	+	+	+	+	+	+
Ear	+	+	+	+	+	+	+	+	+
Eyes	+	+	+	+	+	+	+	+	+
Salivation	+	+	+	+	+	+	+	+	+
Urination	+	+	+	+	+	+	+	+	+
Diarrhoea	_	_	_	_	_	_	_	_	_
Lacrimation	_	_	_	_	_	_	_	_	_
Ataxia	_	_	_	_	_	_	_	_	_
Tremors	_	_	_	_	_	_	_	_	_
Coma	_	_	_	_	_	_	_	_	_
Mortality	_	_	_	_	_	_	_	_	_

*+ Normal – Absent

Table 3: Gross behaviour study of treated animals (Group 3)

Observation	0.5 hr	1 hr	1.5 hr	2 hr	2.5 hr	3 hr	4 hr	1st day	14th day
Respiration	+	+	+	+	+	+	+	+	+
Convulsion	_	_	_	_	_	_	_	_	_
Writhing	_	_	_	_	_	_	_	_	_
Skin & fur	+	+	+	+	+	+	+	+	+
Touch sense	+	+	+	+	+	+	+	+	+
Ear	+	+	+	+	+	+	+	+	+
Eyes	+	+	+	+	+	+	+	+	+
Salivation	+	+	+	+	+	+	+	+	+
Urination	+	+	+	+	+	+	+	+	+
Diarrhoea	_	_	_	_	_	_	_	_	_
Lacrimation	_	_	_	_	_	_	_	_	_
Ataxia	_	_	_	_	_	_	_	_	_
Tremors	_	_	_	_	_	_	_	_	_
Coma	_	_	_	_	_	_	_	_	_
Mortality	_	_	_	_	_	_	_	_	_

*+ Normal – Absent

Table 4: Effect of Triphala extracts on mortality in Swiss Albino Mice

Dose (mg/kg)	No. of animals used	No. of animals died	% of survival
300	6	0/6	100
600	6	0/6	100
1000	6	0/6	100

Body weight analysis

Body weight analysis showed that there is neither increasing nor decreasing body weight of animals between the groups and the data are given as in Fig 4.

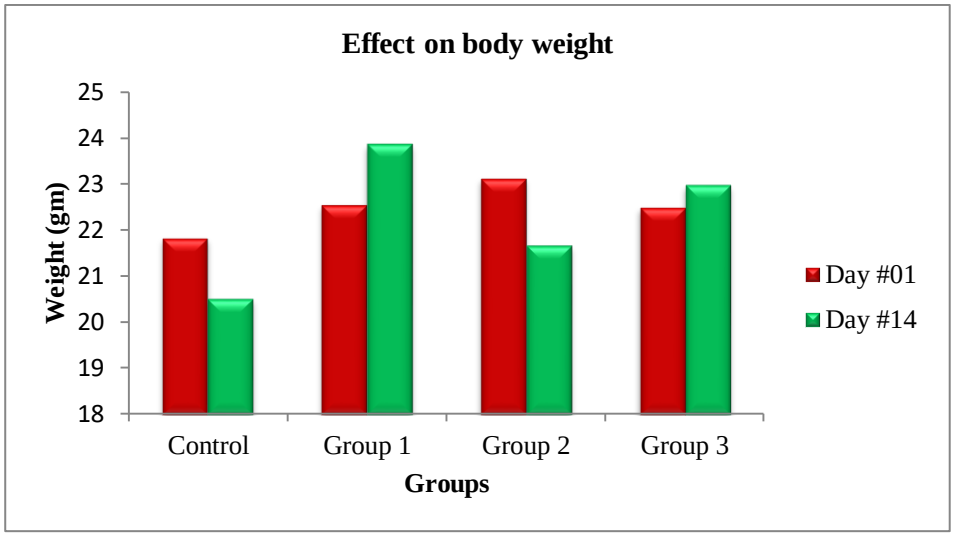

Fig 4: Effect of Triphala extract on body weight of mice

Haematological study

All the haematological parameters of test group were compared to that of the control group and few values were different from those of the control group. Those values were also within the normal ranges. These variations may have ensued from normal fluctuation among animal groups suggesting no immunological defects. Only, the blood glucose level in animals was found to be decreasing with increase in dose of the extract. This is due to the diabetic activity of the phytochemical constituents' present extract. However, there is only very slight decrease in blood glucose level and no significant changes found. The results are given in Fig 5 & 6.

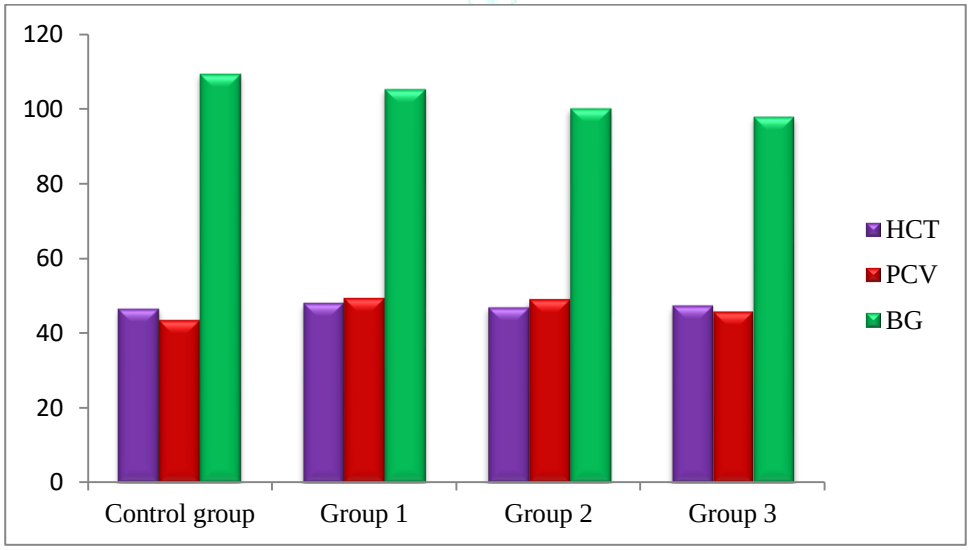

Fig 5: Effect of Triphala extract on haematological parameters (HCT, PCV, BG)

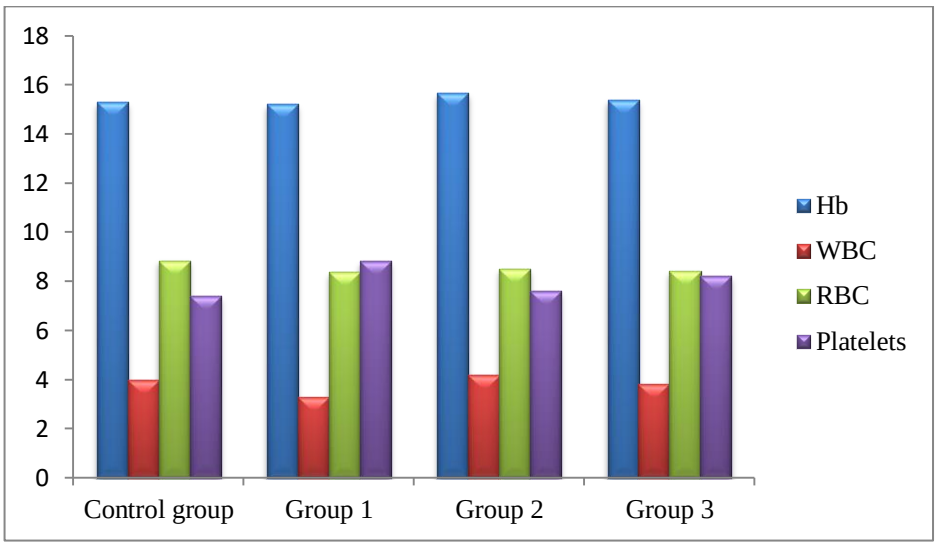

Fig 6: Effect of Triphala extract on haematological parameters (Hb, WBC, RBC, platelets)

Biochemical study

There were no significant changes in the treated group when compared to the control group in the biochemical parameters Serum Glutamic Oxaloacetic Transaminase, Serum Glutamic Pyruvic Transaminase, Triglyceride, albumin, protein, and creatinine. Blood chemical examination was executed in order to assess any toxic effects on liver and kidney. The levels of these blood chemical values have minor changes and remained within the normal range and the results were shown in table 5.

Table 5: Effect on biochemical parameters

Parameters	Control group	Group 1	Group 2	Group 3
SGPT (IU/L)	101.5 ± 0.34	104.8 ± 0.29	103.4 ± 0.42	102.5 ± 0.13
SGOT (IU/L)	278.1 ± 0.61	273.9 ± 0.14	279.6 ± 0.23	275.3 ± 0.35
Albumin (g/dL)	2.97 ± 0.32	3.25 ± 0.56	3.43 ± 0.62	3.12 ± 0.34
Triglycerides (mg/dL)	65.3 ± 0.53	67.4 ± 0.24	69.7 ± 0.13	66.3 ±0.32
Protein (g/dL)	5.63 ± 0.13	5.29 ± 0.32	6.05 ± 0.76	5.87 ± 0. 43
Creatinine (mg/dL)	0.32 ± 0.03	0.38 ± 0.04	0.37 ± 0.01	0.34 ± 0.04

The results are expressed as mean ± S.D

Gross necropsy and histopathological studies

Gross necropsy study was accomplished and the morphological observation of abdominal cavity and their contents, organs like liver and kidney were examined thoroughly and found that there was no sign of inflammation in all the groups.

Histopathological examinations were performed to further confirm whether or not the organs or tissues had been damaged. The results showed no macroscopic or microscopic changes in these internal organs or tissues in any treated animals.

Histopathological sections of liver in control group & treated groups at a dose of 300 mg/kg, 600 mg/kg and 1000 mg/kg showed normal lobular architecture. The portal tracts, sinusoids, central veins, and hepatocytes are found to be normal. No evidence of toxic signs observed as there is no inflammation, fatty change or fibrosis.

The cellular architecture of the kidney of the control and treated groups are studied by the histopathological analysis. Normal cortex, medulla and normal glomeruli were observed from the histological sections of kidney from control and treated animals. Section also showed normal interstitium and no inflammation or necrosis observed.

In-vivo laxative activity of the Triphala extract

Our investigation on laxative activity of Triphala churna shows that the extracts have the ability to increase the bowel movement in constipation condition induced by loperamide. The effect of faecal output in Wistar Albino rats were shown in table 6 and figure 7. Among all the five groups of rats, group 3 rats showed the highest amount of faecal output. The group 4 rats have slight increase in the amount of faecal output when compared to group 2. The group 5 rats has higher amount of faecal output when compared to group 4 but less than that of the group 3. This shows that increasing amount of extract increased the bowel movements in rats which in turn increased the total faecal output.

Table 6: *In-vivo* laxative activity of Triphala extract

Treatment groups		Faecal output (gm)			Total faecal output (gm)
		0 - 8 hours	8 – 16 hours	16 – 24 hours	
Group 1	Normal control	0.649 ± 0.013	0.331 ± 0.025	0.117 ± 0.041	1.093
Group 2	Negative control	0.208 ± 0.037	0.156 ± 0.062	0.109 ± 0.023	0.473
Group 3	Positive control	1.841 ± 0.019	1.124 ± 0.023	0.711 ± 0.024	3.671
Group 4	50 mg/kg of TE	0.712 ± 0.026	0.562 ± 0.086	0.322 ± 0.014	1.595
Group 5	100 mg/kg of TE	1.463 ± 0.072	0.957 ± 0.034	0.571 ± 0.036	2.991

Values are expressed as the mean + standard error of mean (n=6). P < 0.05 compared to control group (One-way analysis of variance followed by Dunnett's multiple comparison test).

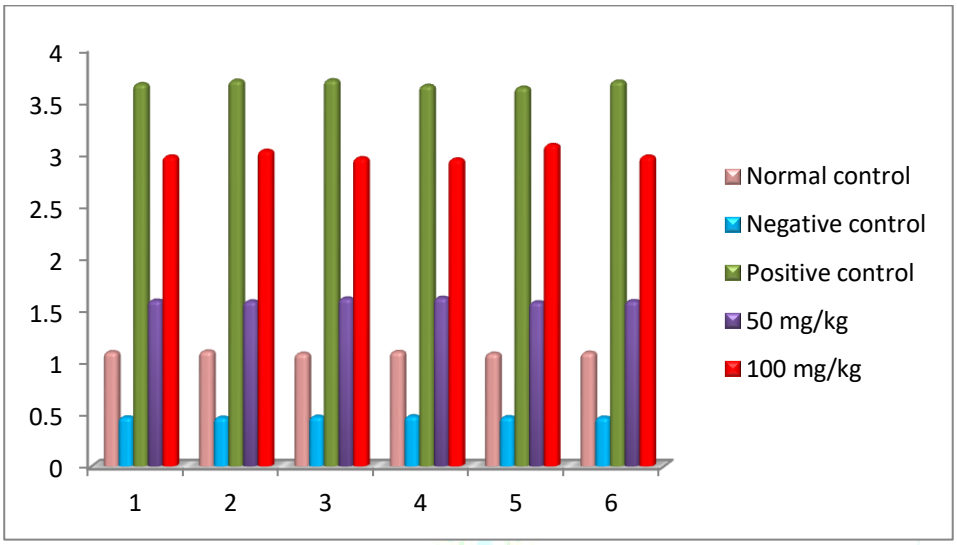

Fig 7: Faecal output of individual animals in all groups (laxative activity)

CONCLUSION

The acute toxicity studies against methanolic Triphala extract on Swiss Albino mice reveals that it is safe to take dosage up to 1000mg/kg since there is no mortality or any gross behavioural changes observed. There is no significant change in body weight, haematological & biochemical parameters except the slight decrease in blood glucose level. This may be due to the effect of antidiabetic activity of Triphala in experimental animal. The laxative activity on Albino Wistar rats shows that the methanolic Triphala extract has significant positive effect on constipated animals.

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