Open Access
Full Length Article

ORIGNAL ARTICLE

Evaluation of Efficacy and Toxicity of Poly Herbal Lozenges in Experimental Animals

Hina Rehman*[*1], Zeeshan Ahmed Shaikh[1], Safila Naveed[2], Muhammad Ibrar Shinwari[3], Khan Usmanghani[1, 2]

[1] Research and Development Department, Herbion Pakistan (Pvt.) Limited, Karachi, Pakistan.

[2] Jinnah University for Women, Karachi, Pakistan.

[3] Department of Environmental Sciences,Faculty of Basic & Applied Sciences,International Islamic University,Islamabad Pakistan.

Keywords: *Poly herbal lozenges, cough, toxicity testing, Efficacy, Lipid profiles, Biochemical toxicity.*

Author`s Contribution
All the authors contributed significantly to the research that resulted in the submitted manuscripts

Article info.
Received: Feb 13, 2018
Accepted: Mar 25, 2018

Funding Source: *Nil*
Conflict of Interest: *Nil*
Cite this article: *Rehman H, Shaikh A, Naveed S, Usmanghani K .* Evaluation of efficacy and toxicity of poly herbal lozenges in experimental animals*. RADS J. pharm. pharm. sci. 2018;6(1):22-32.*

Address of Correspondence
Author: *drhinarehman@hotmail.com*

ABSTRACT

Objective: The study focused on efficacy and toxicity of poly herbal Linkus lozenges in experimental animal.

Methodology: The Acute toxicity test for Linkus lozenges was performed on Albino rats Strain Haffkine with both Sex ratio 1:1.Weight was between 150 – 250 g and ages were 4 – 6 months.0.21 g / kg is the therapeutic dose of the preparation. The morphological examination of organs and estimation of biochemical parameters were assessed for acute and chronic testing. Recommended adult dose was 15 g/day, Dose /kg of body weight was 0.21 g/kg however the therapeutic dose of the preparation was20 (4g/kg) and was tested for chronic toxicity. Housed condition was 25˚C ± 1˚C and 12 h dark/light cycle and food at labitium. In all animals, the drug was administered orally at the dose of 4 g/kg. The animals were kept for acute and chronic toxicity under observation for 2 month and 48 hrs.

Results: No changes in elements of respiratory cardiovascular focal sensory system, gastrointestinal and excretory framework were seen. Average values of different biochemical parameters in test groups were comparable to average values of respective biochemical parameters in control group of animals and found satisfactory however no mortality and abnormality were observed.

Conclusion: No mortality and no abnormality were seen in Linkus lozenges and suggested to be the safe choice.

INTRODUCTION

Cough and sore throat are the frequent symptoms of respiratory disorders and usually annoying and exhausting the patients. Cough is a symptom which cleans the foreign materials and mucus from the upper respiratory track. Due to coughing some mucus or phylum come out from mouth, if not treated well it worsens the normal daily life. If not treated well it causes multiple problems including headache, vomiting, chest pain etc. [1].Continuous coughing creates difficulty in speaking and cause discomfits [2]. Literature studies proves that the herbal and complementary medicine provide a treatment for cough and related symptoms with safety [3, 4].The cause of not practicing herbal plants and

medicines is the lack of pre-clinical data accessibility [5].Many medicinal plants such as Emblica officinalis [6], Asparagus racemosus [7],Trichodesma indicum [8], Asparagus racemosus [9],Zingiber officinale [10],Glycyrrhiza glabra [11],Adhatoda vasica [12],Passiflora incarnate[13],Ocimum sanctum [14] have been reported for anti-tussive activity.

For anti-tussive activity many *in-vitro* testing on whistler rats has been established [15].Many other studies found herbal medicines to be significantly useful in case of treating cough and related symptoms [16]. Commonly the general concept about herbal and complimentary medicines was lack of safety and efficacy data. Misunderstanding is the element which has been created due to lack of data but during last 12 or more years the answers against the misfortune has been established for cough in experimental animals. Numerous consumers of herbal and complementary medicines have treated many diseases including diabetes, auto immune disease and other chronic disease etc. [17, 18].Many centuries herbal medicine is considered to be effective but the authenticity of the efficacy and toxicity should be mentioned as Atropa belladonna etc. [19].

In current study we evaluate the efficacy of Linkus lozenges /extract for cough in comparison with 2 combinations including i.e. Diphenhydramine 8mg/5ml, Acefyllin Piperazine:45mg/5ml andDextromethorphan 6.25mg/5ml, Diphenhydramine 5mg/5ml.After proven efficacy the toxicity has been established with biochemical and hematological parameters on albino wristlet rats.

Linkus lozenges are claimed to be the effective in cough and sore throat associated with respiratory disorders. A Linkus lozenge is the preparation of six-selected herbal drug. Each of these has proved its pharmacological and medicinal action in primary and secondary literatures. It contains the following ingredients Adhatoda vasica, Glycyrrhiza glabra,Piper longum,Viola odorata,Hyssopus officinalis ,Alpinia galangal .The active alkaloid Vasicine and its anti-oxidation product Vasicinone have shown bronchodilator and antihistamine effects [20,21] In the lung of experimental animals the alkaloids, when given intravenously produces a slight but a persistent bronchodilation. This action is in all probability due to depression of the vagal terminals in the bronchi as it is absent with small doses of pilocarpine [22]. Glycyrrhiza is considered to possess demulcent and expectorant properties [23.It is utilized for catarrh of the upper respiratory tract and in addition for gastric/duodenal ulcers [24].Root in implantation, decoction, concentrate or tablet is helpful as demulcent in incendiary friendship or crabby states of the bronchial tubes, entrails and catarrh of the gastro-respiratory sections, for example, hack, roughness, sore throat, asthma, dysuria and so forth [22].Viola odorata is utilized as a part of intense and constant bronchial asthma, intense and incessant catarrh of the respiratory organs, and frosty indications of the upper respiratory tract. The rhizome is utilized as a part of solution for states of the respiratory organs, especially for dry catarrh and for ailment of the minor joints; furthermore utilized for fever, skin maladies, and irritation of the oral mucosa, apprehensive strain, cerebral pain and sleep deprivation. The herb is utilized inside as a part of society solution for hacks, roughness, and tuberculosis, as an expectorant for throat irritation and bronchitis joined by altered mucous, apprehensive strain, a sleeping disorder and mania [24]. Hyssopus officinalis provides diaphoretic, expectorant and anti-spasmodic effects. Alpinia galangal contains Campheride, galangin and alpinin. Respiration in experimental animals was stimulated in small doses. The important action of the drug presented on the bronchioles. Piper longum is an efficacious plant having excellent expectorant properties and is especially employed for bronchitis and whooping cough. The pre-clinical and toxicological study on Linkus lozenges is necessary to be established for enhancing confidence and

prescriptions. As it was assumed that lozenges is just a candy with no clinical improvement. Comparison with the other brands the high dose Efficacy of Linkus lozenges were proved though it had marketed since 2005. The current study is not only based on efficacy but also the increased market share and recognition as a research based OTC. It's considered to be the first of its kind of study in herbal market .This study established the pre-clinical prove and will give the recommendation of the HCPs with the element of confidence and respect. Linkus lozenges were developed in research and development department, Herbion Pakistan Private Limited to be intended to relieve the cough and related symptoms.

MATERIAL AND METHOD

Preparation of Linkus Extract and lozenges

Herbs were purchased through Inventory network Bureau of the Herbion Pakistan (Pvt.) Restricted, Karachi from the nearby market. All examples were put away in dim at 23˚C. The herbs used in Linkus capsules were morphologically contrasted and the genuine example accessible at QC division of Herbion Pakistan Pvt. Ltd., their points of interest are as per the following, Adhatoda vasica reference No. B18, Glycyrrhiza glabra reference No.M4, Flautist longum reference No. F1, Viola odorata reference No. B2, Hyssopus officinalis reference No.Z1, Alpinia galangal reference No.A2. The various chemicals and reagents were of unadulterated systematic review and acquired from nearby supplier.

Preparation of Extracts

Place Herbs (Barring Mulethi remove) with interim into processor. In the wake of squashing of herbs blend with refined water (D. I.) water in extractor and put pounded herbs and Mulethi remove independently. Mixed and after that heated till simmering. The temperature ought to be between 110 - 120 °C. At that point moderate down the temperature and kept up to 90-100 C for 2.5 hours. After filtration the filtrate was exchanged to Vanish Include methyl paraben and Propyl paraben to the concentrated thick concentrate & mixed for 10 minutes.

Manufacturing

The D.I water, sugar and Fluid glucose were put in a tank via auto measuring and blended by stirrer. Exchanged the substance from measure tank to capacity jacketed tank with high temp water temperature 60 to 80°C.Feed the sugar syrup to the cooker through generation Pump concocted item to 148 to 153°C and gathered at the base up to certain level in blaze off vessel outfitted with divider scrubber stir. Product transferred through the conical shaped valve with micrometer adjustment from flash off vessel to vacuum chamber. Lozenges scrap with flavor should not more than 3% of Lot size. Production pump reached the set number and open the flash off vessel chamber which was automatically closed and vacuum released. Bowl moved down by gravity due to weigh of cooked mass.

Product mass transferred from bowl to kneading plate through pan trolley. Add flavored compound and acid(s) to achieve the hardness of product mass by using the chilled water, talc powder used for de moisture. Transferred the mass to batch roller then pass for roping to Rope sizing machine. The set rope for uni-plast machine was assembled and sized mass was stamped to give shape of lozenges in to lozenges compression machine and maintained .Lozenges were finally passed through cooling tunnel to get the desired hardness.

Efficacy

The efficacy of the Linkus lozenges evaluated by Citric acid and linkus lozenges extracts. First cough was induced by citric acid inhalation for 7 minutes with the ratio of 0.15mg/ml .The mice individually were placed in a closed plexiglass chamber (20 × 10 × 10 cm).The cough reflexes

were counted for 7 minutes and comparedto the cough with citric acid +Linkus extract, Linkus extract +Diphenhydramine and Acefyllin Piperazine and Linkus extract + Dextromethorphan, Diphenhydramine.

Acute Toxicity

The Acute toxicity test of Linkus lozenges was performed on Mice of either sex (20-30 g). The animals were treated with two doses (0.23 g dissolved in 1 ml of distilled water and 0.46 g / ml / kg of body weight of Mice) of tablets, which were administered orally. These doses were selected by considering the dosage of tablet in human adult and children, i.e. one tablet 3-4 times / day (4 tablet / day) and maximum up to eight tablet / day. In another group of animals (Control) 0.9 % saline (0.46 ml / kg of body weight of mice, orally) was given. The animals were continuously observed for 4 hours and changes in various autonomic and behavioral responses were noted. Mice were kept under observation for a further period of 15 days to check their general behavioral mortality. The used animal species were Albino rats, Strain were Haffkine with both sexes male and female with 1:1 ratio. Weights were 150 – 250 g and ages were between 4-6 months.

Animals were fed with the pelleted animal food supplied by animal feed industry. The water was given ad. libidum.

Animals were caged and housed in the animal house of the Research and Development Department, Herbion Pvt. Ltd. for 15 days. They were acclimatized for experimental work purpose and were observed for normal behavior. Body weights between 150-200 g were included with free from any fungal infection or any other disease and no injuries on their body. However the animals with normal physiological functions were included.Body weights below 150 g or above 200 g were considered to be excluded.Pregnant female rats , injured and or fungal infections were also excluded.

Experimental design

Controlled single blind randomized study followed by morphological examination of organs after 48 hours and estimation of biochemical parameters at the end of the study. Initial body weight of each animal was recorded for dose calculation. Recommended adult dose was1 lozenge (2.5 g) 6 times a days Or 15 g/day. Average body weight of an adult person was considered 70 kg however the dose /kg of body weight were 15/70 = 0.21 g/kg.20 times (4g/kg) the therapeutic dose was tested for acute toxicity. The drug was administered orally with an oral syringe, by making the solution with distilled water. The number of animals included in each group was 11.The control group of animals was administered water in rats. The animals were kept under observation for 48 hours. Mortality and other abnormal signs and symptoms of behavior, respiratory system, central nervous system, cardiovascular system, and gastrointestinal system were noted. The animals were observed for their altered immune response by noting the incidence of infections and inflammation and having chances of easy lacerations. The animals were also observed for their abnormal locomotory behavior. At the end of 48 hours body weight of each animal was recorded. The animals were sacrificed under light ether anesthesia. The blood was collected. Different hematological parameters were estimated. The serum was separated and processed for Biochemical parameters. Hematological Parameters estimated included total RBC and WBC count, Hemoglobin (Hb) estimation, Differential cell count , Biochemical estimations, Blood glucose, Serum total protein, Serum cholesterol, Serum SGPT, Serum SGOT, Serum alkaline phosphatase, Total bilirubin, Blood urea, Serum creatinine. The animals were dissected. The viscera of the drug treated groups were compared with the control group of animals for inflammation, bleeding and alterations of organs morphologically. All procedures involving animals were reviewed and approved by the

institutional animal care of Jinnah University for Women. The animal protocol was designed to minimize pain or discomfort to the animals.

Chronic Toxicity testing

The animal species for chronic toxicity testing were including Albino rats, strain Haffkine with Sex ratio 1:1 with weight between 150 – 200 g and ages between2 – 3 months. Body weight between 150-200 g, free from any fungal infection and other diseases and injuries on the body were included. Body weight below 150 g or above 200 g, Injuries on their body or with fungal infections was excluded. The animals with abnormal respiratory, cardiovascular, gastrointestinal, excretory and central nervous system functions were excluded from the study. The animals were also observed for their abnormal behavior and excluded from the study. The animals were caged and housed in the animal house of Department of Pharmacology, Faculty of Pharmacy, Karachi University, for 15 days. They were estimated for experimental work purpose and were watched for normal behavior.

Experimental design

Controlled single blind randomized study followed by morphological examination of organs and estimation of biochemical parameters at the end of the study. Initial body weight of each animal was recorded for dose calculation. Recommended adult dose was 1 (2.5 g) 6 times a days or 15 g/day, Average body weight of an adult person considered to be 70 kg and Dose /kg of body weight were 0.21 g/kg as therapeutic dose.20 (4g/kg) times the therapeutic dose was tested for chronic toxicity. The drug was administered orally with an oral syringe. The drug was given daily up to 2 months. The control group of animals was given distilled water. Body weight of animals was recorded every 15 days up to 2 months. Animals were kept under constant observation. Mortality, if any was recorded. Any abnormal sign & symptoms of behavior, respiratory system, central nervous system and cardiovascular system were noted. The animals were observed for their altered immune response by noting infections and inflammation. At the end of the 2 months, animals were sacrificed under light ether anesthesia. The blood was collected by extravasation after decapitation. The serum was also tested for Lipid profile& Biochemical parameters included Serum Lipid Profile, Biochemical Parameters and Hematological Estimations.

RESULTS

The Linkus lozenges efficacy was developed on 3 different models including citric acid +Linkus extract with both sex. Citric acid was used to developed cough reflexes and then minimize by Linkus syrup as shown in table 1.

Table 1:1g/kg citric acid + linkius extract on female rats

Animals	Cough Reflexes						
	Control	10mg/kg	20mg/kg	50mg/kg	100mg/kg	200mg/kg	300mg/kg
1	129	-	220	204	167	147	141
2	235	170	141	197	181	161	153
3	254	227	260	201	144	189	127
4	241	263	201	120	169	137	106
5	194	277	128	206	172	190	184
Mean	**211**	**234**	**190**	**186**	**167**	**165**	**142**

However the reference drug was a combination of Diphenhydramine with Acefyllin Piperazine and citric acid for cough reflexes in male rats were shown in table 2

Table 2: 1g/kg citric acid+ diphenhydramineand acefyllin piperazine

Animals	Cough Reflexes		
	Control	150µl/kg	500µl/kg
1	129	208	131
2	235	304	262
3	254	177	226
4	241	243	141
5	194	212	166
Mean	**211**	**229**	**185**

The cough reflexes on Male rats with citric acid Dextromethorphan and Diphenhydramine in table 3 and 4.

Table 3:1g/kg citric acid + linkus extract

Animals	Cough Reflexes						
	Control	10mg/kg	20mg/kg	50mg/kg	100mg/kg	200mg/kg	300mg/kg
1	240	294	203	154	138	153	185
2	255	191	284	187	158	163	101
3	248	134	222	250	175	173	105
4	129	150	191	164	151	164	99
5	264	262	152	227	106	182	173
Mean	**227**	**206**	**210**	**196**	**146**	**167**	**133**

Table 4: Citric acid + dextromethorphan, diphenhydramine

Animals	Cough Reflexes	
	150µl/kg	500µl/kg
1	266	164
2	181	131
3	235	172
4	187	103
5	266	111
Mean	**227**	**136**

The cough reflexes induced by citric acid were reduced in both sexes with the help of Linkus syrup. The acute and chronic toxicity has been observed on different body weights, biochemical parameters and hematological parameters in table 5, 6 and 7.

Table 5: Effect of linkus lozenges on body weight (dose 4 g/kg)

CONTROL GROUP			EXPERIMENTAL GROUP		
ANIMAL	INITIAL WEIGHT (g)	WEIGHT AFTER 48 HRS OF ADMINISTRATION	ANIMAL	INITIAL WEIGHT (g)	WEIGHT AFTER 48 HRS OF ADMINISTRATION
1	245	243	1	223	222
2	248	245	2	225	225
3	219	221	3	236	235
4	243	240	4	241	242
5	233	233	5	210	212
6	242	241	6	229	230
7	230	229	7	233	232
8	239	239	8	226	226
9	210	211	9	239	238
10	225	225	10	235	236
11	211	210	11	231	231
Mean	231.36	230.64	Mean	229.82	229.91
S.D.	12.39	11.06	S.D.	9.20	8.83
S.E.M.	3.74	3.34	S.E.M.	2.77	2.66

Table 6: Effect of linkus lozenges on biochemical parameters (dose 4 g/kg)

PARAMETERS	CONTROL GROUP	EXPERIMENTAL GROUP	P VALUE
Glucose % g	87.2 ± 3.21	86.78 ± 2.26	-
Total protein g/dl	5.55 ± 0.18	5.96 ± 2.16	-
Cholesterol	82.56 ± 4.96	86.96 ± 5.96	-
SGPT U/l	15.96 ± 12.63	17.86 ± 6.96	-
SGOT U/l	22.96 ± 1.11	25.96 ± 4.96	-
Alkaline phosphatase	19.74 ± 14.85	18.63 ± 11.74	p<0 .01
Urea mg/dl	30.63 ± 6.96	32.85 ± 4.63	-
Creatinine mg/dl	0.86 ± 1.23	0.91 ± 2.96	-
Total bilirubin mg/dl	0.88 ± 0.02	0.87 ± 0.36	-

n=11

Table 7: Effect of linkus lozenges on hematological parameters (dose 4 g/kg)

PARAMETERS	CONTROL GROUP (MEAN ± S.E.M.)	EXPERIMENTAL GROUP (MEAN ± S.E.M.)
Hb %	15.23 ± 0.36	14.63 ± 1.25
Total RBC count mg/dl	8.56 ± 2.366	7.98 ± 4.63
Total WBC count mg/dl	12.58 ± 4.52	13.52 ± 1.2
Neutrophils %	70.12 ± 5.26	69.75 ± 4.63
Lymphocytes %	25.63 ± 1.63	24.63 ± 1.63
Eosinophil %	2.12 ± 1.26	2.63 ± 5.36
Monocytes %	2.23 ± 4.12	2.63 ± 4.96
Basophil %	0.23 ± 4.63	0.22 ± 4.13

Table 8: Chronic toxicity effect of linkus lozenges on body weight

CONTROL GROUP		EXPERIMENTAL GROUP	
INITIAL WEIGHT (G) (MEAN ± S.E.M)	WEIGHT AFTER 2 MONTHS OF ADMINISTRATION (MEAN ± S.E.M)	INITIAL WEIGHT (G) (MEAN ± S.E.M)	WEIGHT AFTER 2 MONTHS OF ADMINISTRATION (MEAN ± S.E.M)
179 ± 0.23	227 ± 0.36	190 ± 1.41	243 ± 0.96

In Chronic toxicity testing no abnormality was observed in the animals when drug was administered for 2 months. The function of respiratory system, cardiovascular system, Central nervous system and gastrointestinal system were normal. No abnormal behavior of the animals was noted. Biochemical, lipid profile & hematological parameters have not significantly altered, thereby confirming the safety of the drug. The increase in the weights of spleen and skeletal muscles are statistically significant. There was no statistical significant change in body weights of the animal. The functions of central nervous system, cardiovascular system, respiratory system, gastrointestinal system and excretory system were all normal. No abnormalities in the behavior of the animals were seen. Different biochemical parameters estimated in rats belonging to test group were complete to respective biochemical parameters in the control group of rats. No mortality was seen as shown in tables below

Table 9: Average weight of body organs

BODY ORGAN	CONTROL GROUP (MEAN ± S.E.M)	EXPERIMENTAL GROUP (MEAN ± S.E.M)
Heart	0.61 ± 0.21	0.72 ± 4.63
Liver	4.53 ±0.36	6.13 ± 5.13
Spleen	0.346 ± 2.50	* 0.64 ± 4.13
Kidney	1.32 ± 4.63	1.42 ± 0.42
Adrenals	0.12 ± 1.63	0.15 ± 3.46
Skeletal muscles	0.46 ± 3.95	* 0.76 ± 1.46

n = 11,Average value ± S.E.M,* $p < 0.05$ as compare to control,** $p < 0.005$ as compare to control

Table 10: Comparison of toxic effects on lipid profile

PARAMETERS	CONTROL GROUP	EXPERIMENTAL GROUP
Cholesterol mg/dl	64.25 ± 2.63	65.63 ± 4.64
Triglycerides mg/dl	22.63 ± 5.86	21.63 ± 4.10
HDL mg/dl	46.23 ± 1.45	46.85 ± 4.66
VLDL mg/dl	4.20 ± 6.79	4.35 ± 4.85
LDL mg/dl	14.96 ± 4.13	15.94 ± 6.13

n = 7,Average value ± S.E.M,* $p < 0.05$ as compare to control,** $p < 0.005$ as compare to control

Table 11: Comparison of biochemical toxicities

PARAMETERS	CONTROL GROUP	EXPERIMENTAL GROUP
Blood glucose mg/dl	56.63 ± 2.63	57.65 ± 4.62
Bilirubin mg/dl	0.62 ± 0.21	0.63 ± 0.42
Alkaline phosphatase U/l	9.97 ± 5.62	9.78 ± 4.16
SGPT U/l	33.65 ± 4.78	32.95 ± 10.23
Creatinine mg/dl	0.89 ± 1.23	0.87 ± 4.62
Total protein g/dl	5.46 ± 4.13	5.56 ± 5.79
Urea mg/dl	29.46 ± 7.63	30.62 ± 8.54

n = 11,Average value ± S.E.M,* $p < 0.05$ as compare to control,** $p < 0.005$ as compare to control

Table 12: Comparison of hematological toxicities

PARAMETERS	CONTROL GROUP	EXPERIMENTAL GROUP
Hemoglobin mg/dl	15.96 ± 5.63	15.45 ± 7.62
Total RBC count	8.36 ± 8.62	9.12 ± 10.21
Total WBC count	9.5 ± 11.21	9.45 ± 5.84
Differential WBC count (%)		
Neutrophil	29.33 ± 2.63	28.52 ± 8.45
Lymphocytes	65.23 ± 5.96	64.85 ± 12.63
Esinocytes	5.16 ± 4.75	5.98 ± 16.52
Monocytes	2.0 ± 1.23	2.67 ± 7.95
Basophils	0.62 ± 7.65	0.76 ± 10.89

n = 11,Average value ± S.E.M,* $p < 0.05$ as compare to control,** $p < 0.005$ as compare to control

DISCUSSION

The Linkus lozenges containing Adhatoda vasica, Glycyrrhiza glabra, Piper longum, Viola odorata, Hyssopus officinalis, Alpinia galanga, were studied for their acute and chronic toxicity in Albino rats. In all animals, the drug was administered orally at the dose of 4 g/kg. The animals were kept under observation for 48 hrs. For chronic toxicity testing body weight was counted for 2 months with given dose of 4g/kg.

After treatment there were no abnormalities observed in behavior of the animals. There was no statistical significant change in body weights of the animals. The functions of central nervous system, cardiovascular system, respiratory system, gastrointestinal system and excretory system were all normal. No abnormalities in the behavior of the animals were seen. Different biochemical parameters estimated in rats belonging to test group were complete to respective biochemical parameters in control

group of rats. No mortality was seen. Average values of different biochemical parameters in test group were comparable to the average values of respective biochemical parameters in control group of animals. The Linkus lozenges are safe when given orally at the Dose of 4 g/kg, which is 20 times the normal therapeutic dose.

The efficacy of linkus lozenges was established by inducing cough with citric acid 1g/kg. On 100mg/kg extract the cough reflexes were 167 as shown in table 1. With citric acid induction with the remedy of Diphenhydramine and Acefyllin Piperazine found to be 229 as shown in table 2. Male albino wristlet rat on 150µg/kg with Dextromethorphan and Diphenhydramine were found to be 227 and Linkus were 167 as shown in table 4. The Linkus extract were more efficacious as compared to the other formulation as mentioned in table 1-4.

There is an expanding utilization of natural or customary prescription everywhere throughout the developing nations because of their prevalence and security on long haul use [25]. Despite the fact that home grown drugs are being utilized for a considerable length of time, in today's world, there are security issues that these meds could conceivably deliver impact on liver, mind or kidneys and subsequently cause irregularity [26]. There are different home grown solutions accessible, however not very many have been taken for clinical and preclinical trial studies to affirm their security and adequacy. The behavioral analysis has proven the safety however toxicity testing the body weights of essential organ for acute toxicity on Linkus extract vs. control were found significant safe as shown in table 5 and the biochemical parameters and hematological parameters were also significantly safe as shown in table 6 and 7 and alkaline phosphate value was<0.01.While the chronic toxicity was significantly safe as compared to Linkus vs. control i.e. 243±0.96,227±0.36 as shown in table 08.The average organ weight including spleen and skeletal muscles were found to be significant <0.05 as shown in table 09.The toxic effect as compare to control considered to be safest as shown in table 10 While the biochemical and hematological parameter has proven the chronic toxicity considered to be the safest and no mortality and physical unevenness shown .

CONCLUSION

The Linkus lozenges, a poly herbal combination were studied for its acute toxicity in Albino rats. The drug was administered orally at the dose of 4 g/kg. After treatment there were no abnormalities observed in behavior of the animals. Also no changes in functions of respiratory system, cardiovascular system, central nervous system, gastrointestinal system and excretory system were seen. Average values of different biochemical parameters in the test group were comparable to average values of respective biochemical parameters in control group of animals. The Linkus lozenges are safe when given orally at the Dose of 4 g/kg, which is 20 times the normal therapeutic dose

REFERENCES

1. *Irwin RS, Madison JM. The diagnosis and treatment of cough. New England Journal of Medicine. 2000, 343(23):1715-21.*
2. *French CL, Irwin RS, Curley FJ, Krikorian CJ. Impact of chronic cough on quality of life. Archives of Internal Medicine. 1998, 158(15):1657-61.*
3. *Irwin RS, Boulet LP, Cloutier MM, Fuller R, Gold PM, Hoffstein V, Ing AJ, McCool FD, O'Byrne P, Poe RH, Prakash UB. Managing cough as a defense mechanism and as a symptom: a consensus panel report of the American College of Chest Physicians. Chest. 1998,114(2):133S-81S.*
4. *Salawu OA, Chindo BA, Tijani AY, Obidike IC, Salawu TA, Akingbasote AJ. Acute and sub-acute toxicological evaluation of the methanolic stem bark extract of Crossopteryx febrifuga in rats. African Journal of Pharmacy and Pharmacology. 2009;3(12):621-6.*
5. *Angell M, Kassirer JP. Alternative medicine—the risks of untested and unregulated remedies.*
6. *Nosal'ova G, Mokrý J, Hassan KT. Antitussive activity of the fruit extract of Emblica officinalis Gaertn.(Euphorbiaceae). Phytomedicine. 2003;10(6-7):583-9.*

7. *Mandal SC, CK AK, Lakshmi SM, Sinha S, Murugesan T, Saha BP, Pal M. Antitussive effect of Asparagus racemosus root against sulfur dioxide-induced cough in mice. Fitoterapia. 2000;1(6):686-9.*
8. *Srikanth K, Murugesan T, Kumar CA, Suba V, Das AK, Sinha S, Arunachalam G, Manikandan L. Effect of Trichodesma indicum extract on cough reflex induced by sulphur dioxide in mice. Phytomedicine. 2002 9(1):75-7.*
9. *Mandal SC, CK AK, Lakshmi SM, Sinha S, Murugesan T, Saha BP, Pal M. Antitussive effect of Asparagus racemosus root against sulfur dioxide-induced cough in mice. Fitoterapia. 2000,1(6):686-9.*
10. *SUEKAWA M, ISHIGE A, YUASA K, SUDO K, ABURADA M, HOSOYA E. Pharmacological studies on ginger. I. Pharmacological actions of pungent constituents,(6)-gingerol and (6)-shogaol. Journal of pharmacobio-dynamics. 1984;7(11):836-48.*
11. *Nawaz A, Bano S, Sheikh ZA, Usmanghani K, Ahmad I, Zaidi SF, Zahoor A, Ahmad I. Evaluation of Acute and Repeated Dose Toxicity of the Polyherbal Formulation Linkus Syrup in Experimental Animals. Chinese Medicine. 2014;5(04):179.*
12. *Dhuley JN. Antitussive effect of Adhatoda vasica extract on mechanical or chemical stimulation-induced coughing in animals. Journal of Ethnopharmacology. 1999;67(3):361-5.*
13. *Dhawan K, Sharma A. Antitussive activity of the methanol extract of Passiflora incarnata leaves. Fitoterapia. 2002;73(5):397-9.*
14. *Nadig P, Laxmi S. Study of anti-tussive activity of Ocimum sanctum Linn in guinea pigs. Indian journal of physiology and pharmacology. 2005;49(2):243.*
15. *Nadig P, Laxmi S. Study of anti-tussive activity of Ocimum sanctum Linn in guinea pigs. Indian journal of physiology and pharmacology. 2005;49(2):243.*
16. *Gupta YK, Katyal J, Kumar G, Mehla J, Katiyar CK, Sharma N, Yadav S. Evaluation of antitussive activity of formulations with herbal extracts in Sulphur Dioxide (SO2) induced cough model in mice.*
17. *Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Brazilian Journal of medical and Biological research. 2000;33(2):179-89.*
18. *Firenzuoli F, Gori L. Herbal medicine today: clinical and research issues. Evidence-Based Complementary and Alternative Medicine. 2007;4(S1):37-40.*
19. *Shader RI, Greenblatt DJ. Uses and toxicity of belladonna alkaloids and synthetic anticholinergics. InSeminars in psychiatry 1971;(Vol. 3, No. 4, p. 449).*
20. *Chopra RN, Chopra IC, Handa KL, Kapoor LD. Indigenous drugs of India Academic publishers. Calcutta-New Delhi. 1982:306.*
21. *Amin AH, Mehta DR. A bronchodilator alkaloid (vasicinone) from Adhatoda vasica Nees. Nature. 1959;184(4695):1317.*
22. *Nadkarni KM, editor. [Indian materia medica]; Dr. KM Nadkarni's Indian materia medica: with Ayurvedic, Unani-Tibbi, Siddha, allopathic, homeopathic, naturopathic & home remedies, appendices & indexes. 1. Popular Prakashan; 1996.*
23. *Moorthy K, Punitha T, Vinodhini R, Sureshkumar BT, Vijayalakshmi P, Thajuddin N. Antimicrobial activity and qualitative phytochemical analysis of Punica granatum Linn.(PERICARP). Journal of Medicinal Plants Research. 2013;7(9):474-9.*
24. *Walker JB. Evaluation of the ability of seven herbal resources to answer questions about herbal products asked in drug information centers. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 2002;22(12):1611-5.*
25. *Daswani GP, Brijesh S, Birdi JT. Preclinical testing of medicinal plants: advantages and approaches. InWorkshop Proceedings on Approaches towards Evaluation of Medicinal Plants Prior to Clinical Trial 2006 (pp. 60-77).*
26. *Saad B, Azaizeh H, Abu-Hijleh G, Said O. Safety of traditional Arab herbal medicine. Evidence-Based Complementary and Alternative Medicine. 2006;3(4):433-9.*