ORIGINAL ARTICLE

Effect of Herbal Syrup Linkus on Acute and Gross Toxicity and Electrolytes in Blood and Organs

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ABSTRACT:
Objective: To evaluate the effect of herbal syrup Linkus on acute and gross toxicity and electrolytes level in blood and organs.
Materials and Methods: This interventional study was carried out in the department of Pharmacology, Faculty of Pharmacy,University of Karachi after approval from BASR-KU. In this study twenty-eight healthy rabbits of either sex weighing 0.90 kg to 1.2 kg. All animals were equally divided into four groups, one served as control group while, remaining three groups received herbal syrup Linkus at three different doses. Acute toxicity was tested in mice by Lorke's method whereas effect on gross toxicity, blood and organ electrolytes was evaluated in Rabbits.
Results: Herbal syrup Linkus exhibited LD_{50} values greater than 5 gm / kg per 24 hrs. No gross toxicity and mortality was observed during the whole experimental period. Non significant changes were observed in blood and organ electrolytes (Calcium, Potassium and Sodium) level.
Conclusion: Herbal syrup Linkus has produced no toxic effect at the administered doses neither showed any significant change in the electrolytes level in the blood and vital organs.
Keywords: Herbal syrup, Linkus, Cough, LD50, Gross toxicity, Electrolytes.

INTRODUCTION:

Herbal medicine, or phytotherapy, is the science of using herbal remedies for the treatment of diseases. Herbal and other plant-derived remedies have been estimated by the World Health Organization (WHO) to be the most frequently used therapies worldwide. Almost 4 billion people (80 percent of the world's population), use herbal medicines for some aspect of primary health care.[1,2] Therapeutic agents derived from plants include pure chemical entities available as prescription drugs (e.g digitoxin, morphine and taxol), standardized extracts, herbal teas, and food plants. Plant-derived remedies can contain ingredients with potent pharmacological and toxicological properties[3] and can play a major role as drugs and as lead structures for the development of new synthetic molecules.[4,5] Increasing consumption of poorly regulated herbal medicines justify review of inherent safety of these products.[6] Herbal products not regulated as medicines cannot be used as alternative therapies unless they are equally effective as accepted agents. Herbal products have the same potential for unwanted effects as patented drugs. A review of randomized controlled trials evaluating herbal medicine revealed that only 15% of studies provided information on their safety or side effects .[7]

The pharmacist's role in selling herbal products, without taking responsibility for those that may not be safe was questioned vigorously by a former Food and Drug Administration (FDA) commissioner.[8] "Natural" should not be mistaken as synonymous with "safe." All medicinal agents can have potentially unexpected effects including toxicity. A user's age, gender, genetics, nutrition status, concurrent disease states and treatments may influence the risk of unexpected effects. Unexpected effects of herbal medicines may be classified as intrinsic or extrinsic to the compound. Intrinsic effects are due to inherent phytochemicals in the given product, such as predictable dose-related toxicity and interactions, as well as idiosyncratic reactions. Extrinsic effects result such as from misidentification of the plant; lack of standardization; impurities through contamination, substitution, or adulteration; incorrect preparation or dosage and inappropriate labeling.[9] Herbal drugs can cause hypersensitivity reactions from dermatitis to anaphylaxis.[10] Despite the importance of plant discoveries in the evolution of medicine, some regulatory bodies such as the US Food and Drug Administration (FDA) consider herbal remedies to be worthless or potentially dangerous.[11]

The increasing use of herbal medicinal products (HMPs) in the community where people are also receiving prescription medicines suggest that adverse herb-drug interactions may be of significant public health consequence. US Food and Drug administration have highlighted herbal-drug interaction as an important safety issue. Despite these concerns, the evidence

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available to guide practitioners in decision making is complex, and encompasses a range of sources, inclusive of reports/series, in vivo and in vitro drug metabolism studies and in vivo drug interaction studies in healthy subjects and patients.[12]

The allopathic cough syrups are known to cause certain side- effects in patient's e.g. gastro-intestinal disturbance, constipation and dizziness or sedation. In addition to side effects, these cough remedies offer only a temporary relief and later on, the patient may develop an addiction for them. The common antitussives in use today include opioid, codeine, and opiate-like agent dextromethorphan.[13, 14]

In the present study, in search of a better antitussive drug of herbal origin, safety of herbal syrup Linkus is evaluated. Previously antitussive effect of herbal syrup Linkus was evaluated and was found to be equal to dextromethorphan.[15]This study was carried out to evaluate the effect of Herbal syrup Linkus on acute and gross toxicity and electrolytes level in blood and organs.

MATERIALS AND METHODS:

This interventional study was carried out in the department of Pharmacology, Faculty of Pharmacy, University of Karachi after approval from BASR of Karachi University from 2006 to 2007.

Chemicals and Reagents: Herbal syrup Linkus was supplied by Herbion Company, Karachi Pakistan. All the chemicals used were of analytical grade. Reagents for Electrolytes testing were supplied by Merck.

Acute Toxicity: LD 50 is defined as the dose of testing material which produces death in 50% animals within 24 hours. In present study, Lorke's (1983)[16] method has been used that require only 13 animals to give LD 50 values, and it has been proved valid for its accuracy.[17] Estimation of LD_{50} was carried out on mice weighing 20-25g bred at the animal house of HEJ Research Institute of chemistry. Herbal syrup Linkus at the dose of 10, 100 and 1000 mg / kg was administered orally to groups of three mice each. Upon the results of mortality in each group after 24 hrs, 4 mice were administered different doses of Linkus herbal syrup in order to obtain the least and most toxic value and LD_{50} was calculated by geometric mean of these values.

Gross (chronic) toxicity, Mortality and effect on electrolytes:

Animals and Grouping: This study was carried out on Twenty-Eight (28) healthy rabbits of either sex weighing from 0.90 kg to 1.2 kg. All the animals were equally divided into four groups, one served as control while, remaining three groups received Herbal syrup Linkus at three different doses. Before administration of drugs, apparent health of these animals was monitored during the conditioning period under the laboratory environments for a week specifically noticing loss of hair, diarrhea, edema, ulceration, and lack of activity. All the animals were maintained under constant environmental conditions 21±1°C. Standard food and water were available ad libitum until the time of drug testing.

Dosing: Herbal syrup Linkus was administered in normal dose animals at 0.57 ml/kg, (prescribed dose 10 ml 3 to 4 times a day), 5 ml/kg moderate dose and 10 ml/kg high dose for a period of 45 days orally. The control group received normal saline orally equivalent to the volume of respective doses according to their body weight.

Gross Toxicity and Mortality Rate: The Gross toxicity was observed at every one-week interval of administration of drug. The following parameters were observed in different groups of animals kept on Linkus syrup during the total period of experiment: Skin ulceration, hematuria, loss of hair, loss of activity, vomiting, diarrhea, edema, salivation, tremor, and aggressive behavior. The number of animals died during these intervals was also noted.

Sample Collection for Biochemical Testing: Technique of cardiac puncture was used to collect blood samples of about 7cc from these animals after completion of dosing period on 46th day to carry out various biochemical tests. Autopsy was performed at the death of the animal or after random selection, in case of survival at the completion of dosage.

Effect on Electrolytes:

Materials:

Water: Double distilled water in polythene containers was used throughout the work. The purity of water was confirmed by its conductivity value (2×10^{-7}/ohm/cm).

Chemicals:

Acids: Concentrated nitric acid (65%) and perchloric acid (60%) manufactured by BDH Chemicals were used to digest the samples.

Reagents: Highly standard solutions of elements under test were used.

Glassware: The Pyrex glassware was used including beakers, pipettes, volumetric flasks. Sample bottles were soaked out for three days in 20% nitric acid and then washed with redistilled deionized water three times and dried in oven.

Methodology:

Equipment: Flame photometer was used to estimate the concentration of electrolytes, Na, K and Ca in the sample solutions.

Preparations of standard solutions:

All standard solutions were made from the standard solution (1000 ppm) of BDH and further dilutions were made up of desired concentration for each metal.

Sodium and Calcium: To prepare 10,20,50,80,100 and 200 ppm standard solutions, 0.5, 1, 2.5, 4, 5, and 10 ml of stock solution respectively were taken in 50 ml volumetric flasks and made up with deionized distilled water.

Potassium: To prepare 15,20,100,150,250 and 300 ppm standard solutions, 0.75, 1, 5,7.5,10,12.5 and 15 ml of stock solution respectively were taken in 50 ml volumetric flasks and made up with deionized distilled water.

Preparation of Sample solutions:

Sampling: The samples of heart, kidney and liver of control and test rabbits were taken from freshly dissected

animals. The samples were immediately transferred to wash sample containers and weighed. The samples were collected as: Kidney –whole, Liver-whole, Heart-whole

Sample treatment: At the time of treatment, the weighed tissues were transferred to the porcelain china dish, which contain 5 ml concentrated nitric acid followed by 5 ml concentrated perchloric acid. It was heated up to 37^0C, initially at low flame and then strongly heated to evaporate the excess amount of acid. When solution was near to dry and thick, cooled at room temperature. This clear solution was transferred to 50 ml volumetric flask and diluted with distilled deionized water. These prepared solutions were stored and used at the time of analysis.

Statistical Analysis: All the values expressed as the means and standard error to the mean (SEM). Student t-test was applied. Results were considered significant if p value were less than 0.05.

RESULTS:

Acute toxicity (LD50): Herbal syrup Linkus exhibited LD50 values greater than 5 gm / kg per 24 hrs. All animals tolerated doses of Linkus up to 5 gm/ kg per 24 hrs periods.

Effect on Gross toxicity and Mortality rate: No gross toxicity and mortality was observed during the whole experimental period.

Effect on Electrolytes:. Animals at normal, moderate and high doses showed increase in calcium level in blood that is 2.430.22 µg/ml , 2.790.60 µg/ml and 2.840.31 µg/ml respectively in comparison to control animals 2.420.26 µg/ml. Animals at moderate and high doses showed rise in potassium level in blood i.e. 3.421.2 µg/ml and 3.000.46 µg/ml respectively. However animals of normal dose showed decrease in potassium level i.e. 2.390.31 µg/ml in comparison to control animals i.e. 2.880.21 µg/ml. Animals at normal and high doses showed decrease in sodium level in blood i.e. 3.160.71 µg/ml and 2.650.34 µg/ml respectively in comparison to control animals i.e. 3.340.30 µg/ml. The animals of moderate group showed rise in sodium level in blood i.e. 4.091.1 µg/ml in comparison to control animals (Table 1).

Animals at normal and moderate doses showed increase in calcium level in kidney i.e. 161.194.7 and 164.404.4 µg/ml respectively in comparison to control animals i.e. 154.824.2 µg/ml. However, the animals at high dose showed decrease in calcium level in kidney i.e. 154.25.8 µg/ml in comparison to control animals. Animals at normal and moderate doses showed increase in potassium level in kidney i.e. 66.09.6 and 71.472.3 µg/ml respectively in comparison to control animals i.e. 57.834.7 µg/ml. While animals received Linkus high dose showed decrease in potassium level in kidney i.e. 53.53 4.4 in comparisons to control group. Animals at normal and high doses showed decrease in sodium level in kidney i.e. 32.123.9 and 37.654.3 µg/ml respectively in comparison to control animals i.e. 41.393.9 µg/ml. However, the animals at moderate dose showed increase in sodium level in kidney i.e. 41.903.0 µg/ml in comparison to control animals (Table 2).

Animals at normal, moderate and high doses showed increase in calcium level in liver i.e. 156.534.5, 157.014.9 and 158.141.9 µg/ml respectively in comparison to control animals i.e. 156.024.1 µg/ml. Animals at normal and high doses showed decrease in potassium level in liver i.e. 64.97.0 and 61.264.3 µg/ml respectively in comparison to control animals i.e. 66.924.6 µg/ml. While animals received Linkus moderate dose showed increase in potassium level in liver i.e. 72.48.8 µg/ml in comparison to control group. Animals at normal and high doses showed increase in sodium level in liver i.e. 39.80 4.8 and 41.174.6 µg/ml respectively in comparison to control animals i.e. 38.812.5 µg/ml. However, the animals of moderate dose showed decrease in sodium level in liver i.e. 36.880.93 µg/ml in comparison to control animals (Table 3).

Animals at normal, moderate and high doses showed decrease in calcium level in heart i.e. 157.07.0, 149.223.6 and 149.37.4 µg/ml respectively in comparison to control animals i.e. 163.785.7 µg/ml. Animals at normal, moderate and high doses showed decrease in potassium level in heart i.e. 69.36.8, 72.084.0, 66.712 µg/ml respectively in comparison to control animals i.e. 79.88.0 µg/ml. Animals at normal and high doses showed increase in sodium level in heart i.e.41.192.5, 40.792.1 µg/ml respectively in comparison to control animals i.e. 40.742.8 µg/ml. However, the animals of moderate doses showed decrease in sodium level in heart i.e. 34.805.6 µg/ml in comparison to control animals (Table 4). All values were found to be non-significant statistically.

Table: 1
Effect of Herbal syrup Linkus on blood Calcium, Potassium and Sodium levels
(N=28)

Electrolytes	Animal Groups (n=7 in each group)			
	Control	Linkus-ND	Linkus-MD	Linkus-HD
Calcium (µg/ ml)	2.42±0.26	2.43±0.22	2.79±0.60	2.84±0.31
Potassium (µg/ml)	2.88±0.21	2.39±0.31	3.42±1.20	3.00±0.46
Sodium (µg/ml)	3.34±0.30	3.16±0.71	4.09±1.10	2.65±0.34

n=7, Average values S.E.M., ND=normal dose, MD=moderate dose, HD=high dose
P value non- significant

Table: 2
Effect of Herbal syrup Linkus on Calcium, Potassium and Sodium levels in Kidney
(N=12)

Electrolytes	Animal Groups (n=3 in each group)			
	Control	Linkus-ND	Linkus-MD	Linkus-HD
Calcium (µg/ml)	154.82±4.2	161.19±4.7	164.40±4.4	154.2±5.8
Potassium (µg/ml)	57.83±4.7	66.0±9.6	71.47±2.3	53.53±4.4
Sodium (µg/ml)	41.39±3.9	32.12±3.9	41.90±3.0	37.65±4.3

n= 3, Average values S.E.M., ND=normal dose, MD=Moderate dose, HD= High dose
P value non-significant

Table: 3
Effect of Herbal syrup Linkus on Calcium, Potassium and Sodium levels in Liver
(N=12)

Electrolytes	Animal Groups (n=3 in each group)			
	Control	Linkus-ND	Linkus-MD	Linkus-HD
Calcium (µg/ml)	156.02±4.1	156.53±4.5	157.01±4.9	158.14±1.9
Potassium (µg/ml)	66.92±4.6	64.9±7.0	72.4±8.8	61.26±4.3
Sodium (µg/ml)	38.81±2.5	39.80±4.8	36.88±0.93	41.17±4.6

n= 3, Average values S.E.M., ND=normal Dose, MD=moderate dose, HD=high dose
P value non-significant

Table: 4
Effect of Herbal syrup Linkus on Calcium, Potassium and Sodium levels in Heart
(N=12)

Parameters	Animal Groups (n=3 in each group)			
	Control	Linkus-ND	Linkus-MD	Linkus-HD
CALCIUM (µg/ ml)	163.78±5.7	157.0±7.0	149.22±3.6	149.3±7.4
POTASSIUM (µg/ml)	79.8±8.0	69.3±6.8	72.08±4.0	66.7±12
SODIUM (µg/ml)	40.74±2.8	41.19±2.5	34.80±5.6	40.79±2.1

n= 3, Average values S.E.M., ND=normal dose, MD=moderate dose, HD=High dose
P value non-significant

DISCUSSION:

Nature provides the foundation for proper health and well-being in its myriad of herbs containing healing properties. That is why millions of people worldwide seek the safer and greater benefits of herbal drugs, as an alternative to patent or prescription cough medicines. Herbal cough and cold remedies are perfect alternatives to expensive prescription drugs. In spite of this widespread acceptance of herbal products in individual self-care choices, misconceptions exist as to the regulation, safety and efficacy of herbal products.[18] In cases where cough is particularly annoying, but not life threatening, a simple may be useful. There are ranges of over-the-counter medicines that can be helpful in such circumstances. A productive, chesty cough, in which phlegm is coughed up, is treated with an to help loosen the phlegm and make it easier to cough it out from the airways. Expectorants contain ingredients such as guaifenesin, ipecachuana or ammonium citrate. A non-productive, dry, tickly or irritating cough, in which no phlegm is coughed up, is treated with a to reduce the cough reflex. Cough suppressants include pholcodine, dextromethorphan and codeine. Other cough suppressants include simple linctus, glycerin, lemon and honey, which coat and soothe the back of the throat.[19]

Present study was conducted to evaluate the safety of herbal cough syrup Linkus, which is commonly used in patients without any pharmacological and toxicological evaluation. Thus considering the situation herbal syrup Linkus was tested for acute toxicity, gross toxicities, effect on blood and organ electrolytes. Rabbits were selected as experimental animals in present study for evaluation of toxic effects because of several reasons that is biochemical changes produced in rabbits are comparatively similar as observed in humans Secondly sufficient amount of blood samples can be obtained at different stages of experiment and thirdly rabbits are not only easily available but are also easy to handle.[20] The result of acute toxicity test indicates that Linkus syrup possess favorable safety profile following oral administration in mice. Oral LD_{50} value for herbal syrup was greater than 5000 mg/kg that was remarkably higher than LD_{50} values of most frequently used antitussive drugs such as codeine and dextromethorphan, having

oral LD_{50} values 154 and 165 mg/kg in mice respectively.[21] Herbs used in herbal syrup Linkus include viola odorata linn ,piperlongum, glycyrrhiza glabra ,adhatoda vasica, alpinia galangal and hysopus officinalis.[22] In present study, animals were also observed for comparison of gross toxicities such as skin ulceration, hematuria, loss of hairs, loss of activity, vomiting, diarrhea, edema, salivation, tremor and aggressive behavior etc. after the administration of different doses of herbal syrup Linkus during the total period of experiment. The results showed no gross toxicities during the whole experimental period. Moreover, there was no death reported at any dose following administration of syrup for 45 days. These findings suggest that this formulation possess wide therapeutic range and is relatively safe. On electrolytes analysis, it was found that there was non-significant changes in blood calcium, potassium and sodium level at all the three doses of the Linkus administered, similarly there was non- significant electrolyte changes in vital organs that is kidney, liver and heart in animals of either group at all the three doses. A study has evaluated the safety of herbal syrup Linkus at various doses and observed that it did not caused mortality in rats at the given doses of 1 or 5 g/kg. Other signs of toxicity like hair loss and weight reduction were also not observed.[23] Electrolytes are important for the body cells. Electrolytes such as calcium, potassium and sodium allow cells to generate electricity, contract muscles, move water and fluids in the body. In our study this herbal cough syrup is found to be a safe formulation as non-significant changes were observed in blood and organ electrolytes. In Pakistan, people frequently use herbal products especially in the rural areas, because of accessibility, affordability and safety.[24,25] On the basis of our results we can justify that this herbal syrup Linkus is a safe formulation for cough and flu.

CONCLUSION:

Herbal syrup Linkus is a safe formulation for use. It has produced no toxic effect at the administered doses neither showed any significant change in the electrolytes level in the blood and vital organs of animals. This justifies its widespread use in cough, cold, flu and other respiratory ailments.

Data collected in present study is however on a small sample. Studies on large number of animals may be conducted in future to further authenticate the safety of herbal syrup Linkus.

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