

ACUTE ORAL TOXICITY AND ANTI TUSSIVE EFFECT OF KOFASIL SYRUP (POLY HERBAL FORMULATION) ON SO₂ INDUCED COUGH MODEL

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ABSTRACT

Introduction- The herbal drugs mentioned in classics for treatment of airway infections are better than modern drugs like anti-tussive, expectorants, mucolytics etc., as they have no any side effect. **Aim:** To evaluate acute oral toxicity on swiss albino mice and anti tussive effect in So₂ induced cough model of kofasil Syrup (Anti-tussive poly-herbal formulation). **Method:** The present study was conducted according to OECD guideline AOT-425 to know single dose toxicity of Kofasil Syrup on swiss albino mice. IAEC No. for the study is SKPCPER/IAEC/2016-02/02. Each mouse was treated with a single oral dose of 2000 mg/kg of extract in sequence at 48 h intervals. Body weight of all animals was recorded once in a week. The anti tussive effect was evaluated in SO₂ induced cough model in mice by method as describe by Miyagoshi et al., 1986 with modified and simplified. **Results:** Body weight of all animals did not reveal any significant change as compared to vehicle control group. Mortality was not observed in any animal of a group. Kofasil Syrup showed significant decrease in cough bouts in compression to standard treated (Codeine Sulphate) group. **Conclusion:** The study shows that Kofasil Syrup (Anti -tussive poly-herbal formulation) did not produce any toxic effect at dose of 2000 mg/kg. So No-Observed-Adverse-Effect-Level (NOAEL) of Kofasil Syrup is 2000 mg/kg. The significant decrease in cough bouts proves potential anti tussive activity of Kofasil Syrup.

KEYWORDS: Anti-tussive poly-herbal formulation, Acute oral toxicity, Kofasil Syrup, Mortality, Swiss Albino Mice.

INTRODUCTION

Medicinal herbs have always been used as traditional primary healthcare agents, especially in Asian countries. Over the last 20 years, rapid changes have been observed in the popular use of natural products from plant sources for maintenance of health and for alternative therapy, in Western countries.^[1] The general public, patients and consumers are primarily interested in fast access to safe and efficient drugs, as well as in animal welfare. Based on their long-term use by humans one might expect plants used in traditional medicine to have low toxicity.^[2,3] Therefore, it should be emphasized that the traditional use of any plant for medicinal purposes, by no means, guarantees the safety of such plant. This raises concern about the potential toxic effects resulting from the short-term and long-term use of such medicinal plants.^[4]

However, while voluminous pharmacological studies have been conducted to ascertain the subjective traditional uses of various medicinal plants, very few plants have been thoroughly evaluated for their detrimental effect. Reports of efficacy are, very far, more numerous than those on toxicity.^[5,6] There is, therefore, a need to further the investigation of herbal remedies and Phytochemical to incorporate the observations of short and long-term toxicity manifestations and to ensure effectual open communication of such finding.

Coughing is a protective mechanism of the body, through which airway secretions or foreign particles can be expelled from the respiratory tract.^[7] Usually it appears in the cold, but It is also a cardinal symptom of some serious conditions like pulmonary HT, pneumonia, TB or asthma.^[8] Various allopathic drugs like anti-tussive, expectorants, mucolytics etc., are being used for the

treatment of cough which gives symptomatic relief in the most extents. But the involvement of debilitating side effects is major drawback of these drugs. These drugs are also contraindicated in the patients having asthma. Therefore, efforts have been trying to use of natural plants to treat the disease without any harmful effects. Anti-tussive agents are commonly used to treat cough and it protects from dry and painful cough. Traditional treatment of cough is cost effective and free from unwanted effects too. Nowadays usage of herbal drug is increasing tremendously.

There are so many plants available to treat the airway infections like cough, pleurisy, bronchial affections, pneumonia and expectoration. With this approach, numerous poly-herbal formulations have been produced which help in the treatment of different types of cough⁹.

The present study has been conducted to test the acute oral toxicity) on swiss albino mice to develop the NOEL

for the same and Anti tussive effect in SO₂ induced cough model of Kofasil syrup (poly-herbal formulation).

AIM AND OBJECTIVES

- To evaluate acute oral toxicity of Kofasil Syrup (Anti-tussive Poly-herbal formulation) on Swiss Albino Mice.
- To evaluate anti tussive effect of Kofasil Syrup (Anti-tussive Poly-herbal formulation) in SO₂ induced cough in Mice.

MATERIALS AND METHODS

Test Material: The test drug (Kofasil Syrup) was manufactured at Petlad Mahal Arogya Mandal Pharmacy, At. Po. Pipalata, Dist. Kheda, Gujarat, India. All the GMP standards were followed during manufacturing. The detail of Kofasil Syrup is mentioned below;

Table 01: Ingredients of Kofasil Syrup (Each 5 ml contains).

Sl. No.	Name of ingredient	Quantity
1	Ext. Adhatoda vasica	100 mg
2	Ext. Glycyrrhiza glabra	100 mg
3	Ext. Terminalia bellerica	100 mg
4	Ext. Ocimum sanctum	75 mg
5	Ext. Zingiber officinale	25 mg
6	Shuddha Takan	25 mg
7	Hordeum vulgare	25 mg
8	Mentha salvestris	3 mg
9	Syrup Base	Q.S.
10	Colour Ponceau 4R	Q.S.

Method: The present study was performed after obtained permission from IAEC (SKPCPER/IAEC/2016-02/02) as per the CPCSEA, Ministry of Environment, Forest and Climate Change (MoFCC), Government of India.

(A) **Acute oral toxicity:**^[10] It was conducted according to OECD guideline AOT-425 to know single dose

toxicity of test drug on swiss albino mice. All the Animals were kept in proper cages with proper diet and acclimatized prior to dosing. They were divided in different groups. Each mouse was treated with a limit single oral dose of 2000 mg/kg of extract in sequence at 48 h intervals. The dosing detail is mentioned below;

Table 02: Individual animal dosing record.

Expt. Day	Animal No.	Gender	Volume dose(ml)
1 st day	H	M	1
3 rd day	B	M	1
5 th day	T	M	1
7 th day	HT	F	1
9 th day	UM	F	1

Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a total of 14 days for any clinical signs of toxicity or mortality. Body weight of all animals was recorded once in a week.

Expt.: Experiment, Conc.: Concentration, H: Head, B: Body, T: Tail, HT: Head & Tail,

UM: Unmarked, M: Male, F: Female

Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a total of 14 days for any clinical signs of toxicity or mortality. Body weight of all animals was recorded once in a week.

(B) Anti-tussive effect:^[11] This study was performed on SO₂ induced cough in mice to know the anti-tussive effect of test drug. The detail of study is mentioned below.

The female Swiss albino mice having weighing range of 25-35g, age 8-12 weeks old, were kept in standard condition and acclimatized for seven days prior to dosing. They were randomized in to three groups with six animals in each group.

Table 03: Grouping of animals.

Group No.	Group name	Dose	No. of Animal
I	Disease control (DC)	NA	06
II	Standard drug treated (Std.) (codeine sulphate)	10mg/kg	06
III	Kofasil syrup	335mg/kg	06

The antitussive activity against Sulphur dioxide (SO₂) induced cough was evaluated by the method as describe by Miyagoshi *et al.*, 1986^[12] as modified and simplified. A Petri dish containing solution of 2 ml of 500 mg/ml of sodium hydrogen bisulfate (NaHSO₃, S. D Fine-Chemical Ltd.) in double distilled water was placed in one compartment of chamber. After that, 0.2 ml of concentrated Sulphuric acid (H₂SO₄, S. D Fine-Chemical Ltd.) was added in to it by using a pipette to produce SO₂ gas according to reaction as follow;

$$2\text{NaHSO}_3 + \text{H}_2\text{SO}_4 \rightarrow 2\text{SO}_2 + \text{Na}_2\text{SO}_4 + 2\text{H}_2\text{O}$$

After 15 seconds, mice were placed in the other compartment of the chamber and exposed to SO₂ gas for 45 seconds. Then they were taken out from the chamber and put in to a big plate and cough bouts were counted for 5 minutes. In the same manner above procedure was repeated for all the mice of the treated groups and frequency of cough bouts was measured. The drugs were administered orally. The frequency of cough bouts was assessed before and after treatment.

Statistical Analysis: Arithmetic mean and standard error of mean were calculated from the individual observations. The data was expressed as mean ± S.E.M. Statistical difference between two groups is tested by using student's paired t-test. p<0.05 was considered as significant.

OBSERVATIONS AND RESULT

The animals were observed continuously for behavioural changes, autonomic profiles and other signs of toxicity or mortality up to a period of 14 days. The body weight, food intake and water intake were also observed on 1st, 7th and 14th day. There were no physical and behavioural changes observed in swiss albino mice during observation period. Body weight of all animals did not reveal any significant change as compared to vehicle control group. Mortality was not found in any group.

Table 04: Showing individual animal weekly body weight record, dose and mortality.

Animal No.	Sex	Dose (mg/kg)	Experiment Day & Date Unit : gm			Mortality
			1 st	7 th	14 th	
H	M	2000	26	27	28	Nil
B	M	2000	22	23	23	Nil
T	M	2000	24	25	26	Nil
HT	F	2000	27	28	29	Nil
UM	F	2000	28	28	29	Nil

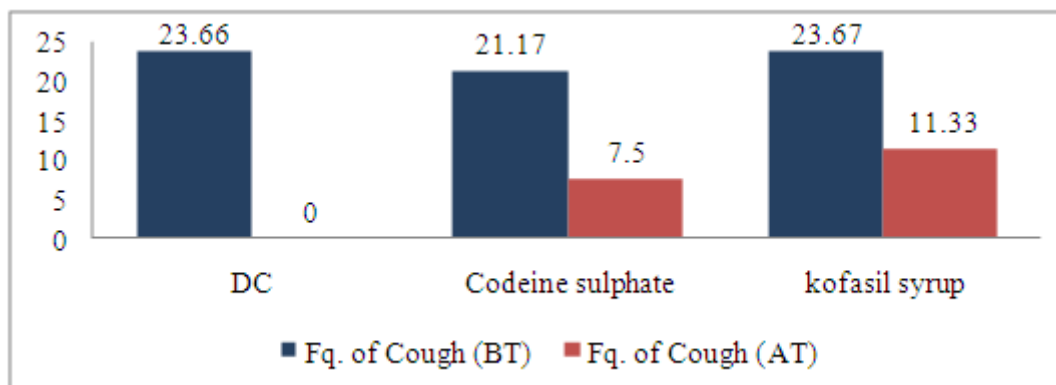
H: Head, B: Body, T: Tail, HT: Head & Tail, UM: Unmarked, M: Male, F: Female

(B) Anti-tussive effect: Sulphur dioxide induced cough model in mice (*Gupta Reena et al, 2014*):

Table 05: Showing Effect of Kofasil syrup on frequency of cough bouts in SO₂ induced cough model in mice (Value of cough bouts are expressed as mean ± S.E.M. (n=6)).

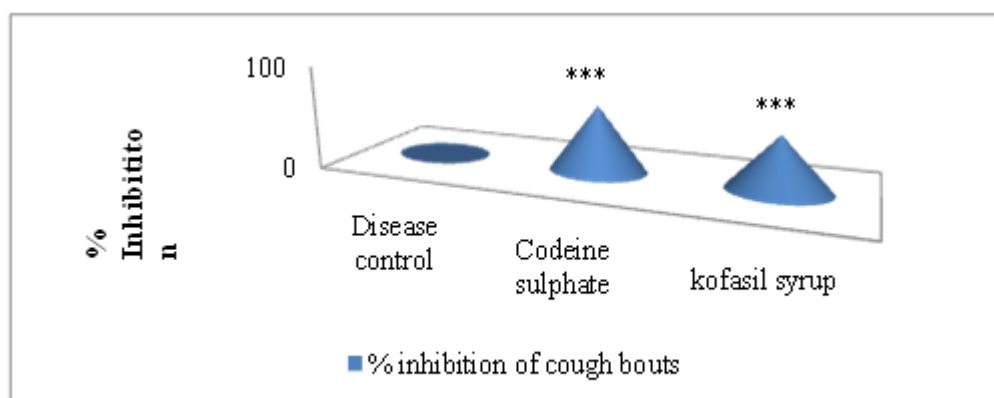
Group No.	Treatment & Dose (mg/kg)	Frequency of cough bouts before treatment mean ± SEM	Frequency of cough bouts after treatment mean ± SEM	% inhibition of cough bouts
I	Disease control	23.66	-	-
II	Codeine sulphate (10 p.o)	21.17 ± 0.94	7.5 ± 0.67	64.55***
III	Kofasil syrup (335 p.o)	23.67 ± 2.01	11.33 ± 1.14	52.11***

***P<0.001 Vs Before treatment.



Graph 1 Frequency of cough bouts before & after treatment

Value of cough bouts are expressed as mean \pm S.E.M. (n=6). ###P<0.001 Vs Normal Control ,***P<0.001 Vs Before treatment.



Graph 2: % Inhibition of cough bouts before & after treatment.

DISCUSSION

The acute toxicity profile of Kofasil Syrup (Anti-tussive poly-herbal formulation) is not available till date. This study can consider as a pioneer step for the establishment of safety profile of Kofasil Syrup (Anti-tussive poly herbal formulation). It also reveals a safe use of this formulation as an effective poly-herbal cough syrup.

This formulation contains ingredients as mentioned in (Table01). *Adhatoda vasica* is used in cough, asthma, cold, bronchitis and tuberculosis.^[13] *Glycyrrhiza glabra* used in cough and tuberculosis management.^[14] *Terminalia bellerica* is rejuvenative and cures cough disorder and beneficial for throat.^[15] *Ocimum Sanctum* is the herb used for common cold, cough and have anti-inflammatory activity.^[16] *Zingiber officinale* is useful in respiratory tract infections. Vomiting.^[17] *Shuddha tankan* used in management of chest congestion, cough, bronchitis and wheezing.^[18] *Hordeum vulgare* is used in conditions like bronchitis and inflammations.^[19] *Mentha salvestries* highly effective in treating headache, rhinitis, cough, sore throat, colic & Vomiting.^[20]

Oral route of drug administration is perhaps the most appealing route for the delivery of drugs. The syrup is advantageous dosage form among the various dosage

forms administered orally because of having more flexibility in achieving the proper dosage of the medicines and helping in faster absorption.

The anti tussive activity of test drug was performed in Sulphur dioxide (SO₂) induced cough model. The test drug treated group shows significant decrease in cough bouts and greater % inhabitation of cough bouts (Table 05) which favors its potential anti tussive effect as compared to DC and Standard drug treated groups. Kofasil syrup showed positive effect to reduce cough, which might be due to reduction of the bronchial irritation and also by suppressing the cough center in medulla.

CONCLUSION

This study reveals that Kofasil Syrup (Anti-tussive poly-herbal formulation) does not have any toxic effect at dose of 2000 mg/kg. So No-Observed-Adverse-Effect-Level (NOAEL) of Kofasil Syrup is 2000 mg/kg. The significant decrease in cough bouts proves potential anti tussive activity of Kofasil Syrup (Anti-tussive poly-herbal formulation).

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REFERENCES

1. Wills RB, Bone K, Morgan M. Herbal products: Active constituents, modes of action and quality control. *Nutr Res Rev.*, 2000; 13: 47-7.
2. V.Ertekin, M. A. Selimoğlu, and S. Altinkaynak, "A combination of unusual presentations of Datura stramonium intoxication in a child: rhabdomyolysis and fulminant hepatitis," *Journal of Emergency Medicine*, 2005; 28(2): 227–228. [4] S.
3. Koduru, D. S. Grierson, and A. J. Afolayan, "Antimicrobial activity of Solanum aculeastrum," *Pharmaceutical Biology*, 2006; 44(4): 283–286.
4. A.N.U kwuani, M.G. Abubakar, S.W. Hassan, and B.M. Agaie, "Toxicological studies of hydromethanolic leaves extract of *Grewia crenata*," *International Journal of Pharmaceutical Science and Drug Research*, 2012; 4: 245–249.
5. Ekor, M. The Growing Use of Herbal Medicines: Issues Relating to Adverse Reactions and Challenges in Monitoring Safety. *Front. Pharmacol.* 2014, 4. [CrossRef] [PubMed] 3. Chalut, 6 D.S. Toxicological risks of herbal remedies. *Paediatr. Child Health*, 1999; 4: 536–538.
6. K. D. Tripathi *Drugs for Cough and Bronchial Asthma*, *Essentials of medical pharmacology*, sec.4, 2003; 16: 213-216.
7. Meenakshi parihar *et al* A Review – cough & treatments, 2011; 1(1): 9-18.
8. Meenakshi parihar *et al* A Review – cough & treatments, 2011; 1(1): 9-18.
9. OECD guideline for Testing of Chemicals, Acute oral toxicity Up and down procedure, OECD – 425, adopted on 17th, December 2001; 1-26.
10. Gupta Reena *et al* Evaluation of Antitussive activity of Polyherbomineral formulation on cough reflex induced by different cough induced models in mice, *Int. Journal of Drug development and Research*, 2014; 6(4): 93-102.
11. Miyagoshi M *et al* Antitussive effects of lephedrine, amygdalin and makyokansokito (Chinese traditional medicine) using a cough model induced by sulfur Dioxide gas in mice, *Planta Med.*, 52: 275-278.
12. [www.ayurveda for you.com/Ayurveda.herb/vasa.html](http://www.ayurvedaforyou.com/Ayurveda.herb/vasa.html).
13. www.webmd.com/vitamins/ai/ingredientmono-881/licorice.
14. [www.iloveindia.com/india herbs / terminalia belerica/html](http://www.iloveindia.com/india_herbs_/terminalia_belerica/html).
15. [http://www.alwaysayurveda.com/occimum sanctum](http://www.alwaysayurveda.com/occimum_sanctum).
16. [www.planet Ayurveda.com/library/ginger.zingiber officinale](http://www.planet Ayurveda.com/library/ginger.zingiber_officinale).
17. [amp/s/ayurveded.info.com/2012/05/22/tankan bhasma benefits](http://amp/s/ayurveded.info.com/2012/05/22/tankan_bhasma_benefits).
18. www.m.webmd.com/vitamins/ai/ingredientmono-799.
19. [www.iloveindia.com/indian herbs/podina](http://www.iloveindia.com/indian_herbs/podina).