Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN **0976-9595** Research Article

Production of Kalomegh Syrup and Studies on Its Toxic Activities

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ABSTRACT

Kalomegh syrup was prepared from ethanolic extract of kalomegh (*Andrographis paniculata*) leaves .The recipe composition and specification of the product has been ascertained. A toxicity test of the product has also been performed on a group of mice. Results revealed that the physical movement and growth of treated and untreated mice are normal. The hematological and bio-molecule parameters like, RBC, WBC, serum glucose, serum *protein*, serum cholesterol, serum creatinine etc. have been measured and found no significant or adverse effect. The product is found to be useful in anti-fever and anti-toxic. It has been uses in case of bacillary dysentery, respiratory infections, tonsillitis, pharyngitis, laryngitis, pneumonia, tuberculosis, nephritis and also for dermatological diseases like crusty tetter and herpes and helminthic intestinal infestation.

Keywords: Andrographis paniculata, etahnolic extract, cytotoxicity, hematological, bio-molecule.

1. INTRODUCTION

Andrographis paniculata belonging to family Acanthaceae, commonly known as Kalmegh is one of the widely used medicinal herb. It is an important drug in ancient system of medicine [1, 2]. Whole plant has a wide range of pharmacological activity [3]. It is a bush, which grows in India, Bangladesh, China, South East of Asia and in West Indies. It reaches a height of 30-100 cm. The geographical distribution of this plant has led to its traditional use in Ayurvedic (India), Thailandese and Chinese medicine. Its principle active compounds are: flavonoids, glycosides and ditherpenic lactones (called andrographolide, bitter compounds in the stem and leaves). The structure of andrographolide is shown below. Its mol. formula: $C_{20}H_{30}O_5$; mol. wt.: 350.4.



The compounds are anti-thrombotic (blood clot preventive), cancerolytic (fight, even kills, cancer cells), cardioprotective, digestive (promotes digestion), hepatoprotective (protects the liver and gall bladder), hypoglycemic (blood sugar reducer), laxative, sedative, thrombolytic (blood clot buster) and vermicidal (kills intestinal worms). It is also effective against a variety of infections and oncogenic agents [4], antiviral [5], common colds [6], antipyretic [7], analgesic (pain killer), anti-inflammatory, antibacterial, anti-malarial [8], anti-diarrheal [9], anti-fertility and other diseases [10]. So, most of Andrographis' traditional uses have scientific basis. Its most significant physiological effect is that of a "signal transducer" that is it stops the unregulated cell growth caused by viruses. Considering its importance in medicinal value, efforts have been made to prepare medicated kalomegh syrup from ethanolic extracts of its leaves. The toxicity test of the prepared kalomegh syrup has been studied on group of Swiss albino mice and found no adverse effect. The present paper deals with the same.

2. MATERIALS AND METHODS

2.1. Collection of plant materials

For the present study fresh leaves of kalomegh (*Andrographis paniculata*) plants were collected from the experimental field of BCSIR Laboratories, Rajshahi.

2.2. Production of kalomegh syrup

The collected leaves (5.0 kg) were washed and then blanched at 45° - 50° C for 10 minutes. The blanched leaves were then dried in an air-circulating oven at $40^{\circ}\pm2^{\circ}$ C. A grinding machine then crushed it into powder form (1 kg; moisture, 5.5%). The powders were immersed in sufficient quantity of

distilled ethyl alcohol at room temperature for 48 hours. The extract was collected and the process of extraction was repeated five times more with fresh ethyl alcohol until the extracts become almost colorless. The extracts were combined and concentrated by removal of the solvent in a rotary evaporator under reduced pressure at a temperature below 45° C. A greenish dense mass (200 gm) was obtained at this stage. To this dense mass sugar solution (3,8 kg), citric acid (10.0 gm) and sodium meta- bisulphite (5.0 gm) were added and mixed well. The whole substance was then heated at 60° - 70° C for 10 minutes. The final product was the desired kalomegh syrup. Yield 4.0 kg (80% on the basis of weight of fresh kalomegh leaves taken).

Recipe composition

1.	Kalomegh powder	1.0 kg
2.	Sugar	2.5 kg
3.	Water	1.5 litre
4.	Citric acid	10.0 gm
5.	Sodium meta-bisulphite	5.0 gm

Specification of the product

Ash content:	1.6%
Total sugar:	65%
Fat:	0.05%
Dietic fibre:	0.6%
Shelf-life:	1 year
Toxicity:	No toxicity was found
Calorific value	300 Kcal

2.3. Organoleptic evaluation of the prepared kalomegh syrup

The prepared kalomegh syrup was presented to a panel consisting of 10 persons and was evaluated to find the consumer acceptability of the product prepared. The organoleptic evaluation was done based on the Hedonic scale.

2.4. Determination of toxicity activity

The toxicity test of the prepared kalomegh syrup has been studied on group of Swiss albino mice. The mice were clinically healthy and were kept under standard environmental conditions of temperature $(27^{0}\pm2^{0}C)$. For the present study, Swiss albino mice were divided into 3 groups of three animals each. Group 1 was given normal diet only and treated as control one. Group 2 was given 1 ml of prepared kalomegh syrup per day with their normal diet and that of group 3 was given 2 ml per day. The effect of kalomegh syrup on physical movement and growth of treated and untreated mice have been observed. The effect of kalomegh syrup on hematological parameters such as red blood cells (RBC), white blood cells (WBC), Lymphocytes, Neutrophil and Monocytes in treated and untreated Swiss albino mice have been measured. The effects of kalomegh syrup on biomolecules such as serum glucose, cholesterol protein and creatinine have also been measured. Experiments showed that the physical movement and growth, hematological and bimolecular parameters of treated mice are normal in comparison to that of untreated mice. The results are summarized in Table 2, Table 3 and Table 4.

3. **RESULTS**

The qualitative chemical examination of ethanolic extract of kalomegh leaves revealed the presence of flavonoids, glycosides and andrographolids. The product kalomegh syrup was prepared by our developed technology. The consumer acceptability data are given in Table 1. It is evident from Table 1 that the prepared kalomegh syrup according to taste panelists was found to be better from colour, taste, flavour, texture, and sweetness point of view. The toxicity activity of the syrup was performed against a group of mice.

Table 2 shows the physical behavior of the treated and untreated mice. In-group 1, each mice are treated with normal diet (5 mg/ day) and their weight was found to vary 27.0- 27.8 (mice 1), 24.8- 26.5 (mice 2) and 25.5- 27.0 gm (mice 3). Ingroup 2, each mice are treated with 1 ml of kalomegh syrup with their normal diet and their weight was found to vary 26.5- 27.7 (mice 1), 25.8- 27.8 (mice 2), 26.0-28.0 gm (mice 3) respectively. Similarly in group 3, each mice are treated with 2 ml of kalomegh syrup with their normal diet and their weight was found to vary 26.2- 26.8 (mice 1), 27.5- 28.2 (mice 2), 26.2-27.5 gm (mice 3) respectively. The results revealed that the weight of treated and untreated mice increases significantly and their physical movements are normal.

From Table 3, it is evident that hematological parameters such RBC, in normal mice (group 1), the RBC count was found to vary $(8.05 \times 10^9) - (9.01 \times 10^9)$ cells/ml. In treated mice (group 2), the RBC count was found to vary (8.03×10^9) $- (9.35 \times 10^9)$ cells/ml. In other treated mice (group 3), the RBC count was found to vary $(8.25 \times 10^9) - (9.10 \times 10^9)$ cells/ml. Similarly, in normal mice (group 1), the WBC count was found to vary $(6.65 \times 10^6) - (7.01 \times 10^6)$ cells/ml. In treated mice (group 2), the WBC count was found to vary $(6.90 \times 10^6) - (7.20 \times 10^6)$ cells/ml. In other treated mice (group 3), the WBC count was found to vary $(6.25 \times 10^6) - (7.02 \times 10^6)$ cells/ml. No significant changes were observed in Hb, lymphocyte, neutrophil and monocyte counts after treatment with the product (Table 3).

On the 7th day of study

The amount of serum glucose in normal mice (group 1) was found to vary 12.60 –13.8 mg/l00ml. The amount of serum glucose after treatment at dose 1 ml (group 2) with kalomegh syrup was found to vary 13.75-14.25 mg/100 ml and at dose 2 ml (group 3) it was found to vary 13.7-14.32 mg/100 ml respectively.

The amount of serum protein was found to vary 14.0-15.20, 15.5-15.95 and 13.70-14.32 mg/l00 ml for group 1, group 2 and group 3 respectively. Similarly, the amount of serum cholesterol was found to vary 55-59, 51-59 and 51-58 mg/l00 ml for group 1, group 2 and group 3 respectively. No significant changes were observed in serum creatinine level after treatment with the product (Table 4).

Table 1. Consumer acceptability trial of kalomegh syrup (meanscore given by 10 persons)

Quality Factors	Results
Colour (1-9)	8.8 Above like verv much
	Below like extremely
Flavour (1-9)	8.6
	Above like very much
	Below like extremely
Texture (1-9)	8.9
	Above like very much
	Below like extremely
Sweetness (1-9)	8.9
	Above like very much
	Below like extremely
Taste (1-9)	9.0
	Like extremely
Overall acceptance	9.0
(1-9)	Like extremely

Eval	uated	on	9	points	hed	onic	scale	e.
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Table 2. Effect	of kalomegh syrup or	n physical behavio	ors on the
first day of stu	dy		

	Mice	Weight	Food	Physical	Growth
		(gm)	consume/mice	movement	
	Mice1	27.0	5gm/day	Normal	Normal
Group-1	Mice2	24.8	5gm/day	Normal	Normal
	Mice3	25.5	5gm/day	Normal	Normal
	Mice1	26.2	5gm/day	Normal	Normal
Group-2	Mice2	25.5	5gm/day	Normal	Normal
1	Mice3	26.0	5gm/day	Normal	Normal
	Mice1	25.8	5gm/day	Normal	Normal
Group-3	Mice2	27.3	5gm/day	Normal	Normal
	Mice3	26.2	5gm/day	Normal	Normal

	Mice	Weight	Food	Physical	Growth	
	whee	(gm)	consume/mice	movement	diowui	
	Mice1	27.2	5gm/day	Normal	Normal	
Group-1	Mice2	25.0	5gm/day	Normal	Normal	
	Mice3	25.8	5gm/day	Normal	Normal	
	Mice1	26.5	5gm/day	Normal	Normal	
Group-2	Mice2	25.8	5gm/day	Normal	Normal	
	Mice3	26.4	5gm/day	Normal	Normal	
	Mice1	26.0	5gm/day	Normal	Normal	
Group-3	Mice2	27.5	5gm/day	Normal	Normal	
	Mice3	26.5	5gm/day	Normal	Normal	

On the 14^{th} of study

	Mico	Weight Food		Physical	Growth	
	white	(gm)	consume/mice	movement	Glowul	
	Mice1	27.5	5gm/day	Normal	Normal	
Group-1	Mice2	25.8	5gm/day	Normal	Normal	
Group-1	Mice3	26.2	5gm/day	Normal	Normal	
	Mice1	27.0	5gm/day	Normal	Normal	
Group-2	Mice2	26.8	5gm/day	Normal	Normal	
	Mice3	27.2	5gm/day	Normal	Normal	
	Mice1	26.5	5gm/day	Normal	Normal	
Group-3	Mice2	28.0	5gm/day	Normal	Normal	
	Mice3	27.0	5gm/day	Normal	Normal	

On the 21st day of study

	Mice	Weight	Food	Physical	Growth
		(gm)	consume/mice	movement	
	Mice1	27.8	5gm/day	Normal	Normal
Group-1	Mice2	26.5	5gm/day	Normal	Normal
	Mice3	27.0	5gm/day	Normal	Normal
	Mice1	27.7	5gm/day	Normal	Normal
Group-2	Mice2	27.8	5gm/day	Normal	Normal
	Mice3	28.0	5gm/day	Normal	Normal
	Mice1	26.8	5gm/day	Normal	Normal
Group-3	Mice2	28.2	5gm/day	Normal	Normal
	Mice3	27.5	5gm/day	Normal	Normal

Group-1 which feed on normal diet

• Group-2 which feed on normal diet with 1 ml/day/mice kalomegh syrup

• Group-3 which feed on normal diet with 2ml/day/mice kalomegh syrup

Group	Mice	RBC	WBC	% of	Lymphocytes %	Neutrophil %	Monocytes %
*		cells/ml	cells/ml	Hb		•	-
	Mice1	9.01×10^{9}	6.8×10^{6}	13.15	73	20	7
Group-1	Mice2	8.05×10^{9}	7.01×10^{6}	12.96	71	21	8
	Mice3	8.11×10 ⁹	6.65×10 ⁶	13.25	72	18	10
	Mice1	8.03×10 ⁹	7.0×10^{6}	13.85	74	17	9
Group-2	Mice2	9.02×10^{9}	6.90×10^{6}	14.02	73	18	9
	Mice3	9.35×10 ⁹	7.2×10^{6}	13.50	72	19	9
	Mice1	8.25×10 ⁹	6.25×10^{6}	11.98	73	19	8
Group-3	Mice2	9.10×10^{9}	6.75×10^{6}	13.80	71	21	8
	Mice3	8.5×10 ⁹	7.02×10^{6}	12.08	74	17	9

Table 3. Effect of kalomegh syrup on hematological parameters in Swiss albino mice on 21st day of study

Table 4. Effect of kalomegh syrup on biomolecules of Swiss albino mice on 21st day of study

Group	Mice	Serum glucose (mg/ml)	Serum protein (mg/ml)	Serum cholesterol (mg/ml)	Serum creatinine (mg/ml)
Group-1	Mice1	13.80	14.4	57	4.5
	Mice2	12.60	15.20	55	5
	Mice3	13.50	14.0	59	4.20
Group-2	Mice1	13.75	15.5	54	4.35
	Mice2	14.02	14.75	51	5.02
	Mice3	14.25	15.95	58	4.10
Group-3	Mice1	13.80	14.20	55	5.05
^	Mice2	14.50	13.70	53	4.55
	Mice3	14.95	14.32	56	4.32

4. CONCLUSION

From this experiment it is concluded that extracts of kalomegh leaves in ethanol solvent have no toxic effect on living organism. The results obtained from the present study provide a strong basis for further studies on searching anticancer, antimicrobial and pharmacological principles from kalomegh leaves in future.

5. ACKNOWLEDGEMENT

The author wishes to acknowledge Mr. Ashrafuzzaman, Department of Applied Nutrition and Food Technology, Islamic University, Kustia for carrying out toxicity activity of the product, kalomegh syrup.

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