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Research Article

Preclinical Toxicity Assessment of UNIM-352, a Polyherbal Unani Preparation, and its Optimized Formulations: A Potential Therapeutic Modality for Bronchial Asthma

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Abstract

Objective: UNIM-352, a polyherbal preparation containing six ingredients *Linum usitatissimum, Trigonella-foenum-graecum, Allium sativum, Strychnos potatorum, Caesalpinia bonducella* Fleming (1g) and *Pongamia glabra*, Honey (q.s.), is used in Unani traditional system of medicine for bronchial asthma.

Method: In an attempt to interactions between traditional and modern medicine, our earlier clinical study using modern scientific methodology showed that UNIM-352 administration improved the efficacy of conventional pharmacotherapy and was well tolerated in the patients of bronchial asthma and thus, could be considered as a viable adjunct for asthma treatment. The safety of UNIM-352 and optimized preparations of UNIM-352 were evaluated by sub-acute oral toxicity studies in rats following OECD guidelines.

Result: The results showed no apparent signs of toxicity and body weight changes compared to that in experimental control group of rats. Mean organ body weight, haematological parameters drug treated groups were within normal biological range.

Conclusion: Histopathological examination of various organs showed no abnormal structural abnormalities. The results of subacute toxicity studies showed that optimized preparations of UNIM-352 were safe upto the dose of 2000 mg/kg.

Keywords: *OECD; UNIM-352; Polyherbal; Histopathology*

Introduction

Traditional use of herbal drugs have long been used and documented. They are generally safe and effective and recognized as a form of alternative medicine in conventional scientific based health system (Ojha and Tiwari, 2014). The World Health Organization (WHO) also promotes and recommends herbal drugs and encourages the use of traditional herbal drugs in National Health Care Programmes because of their low cost, easy availability, faith of the people in such remedies and safety [1]. Since ancient times, various plants and plant derived products have been used traditionally for the treatment of bronchial asthma throughout the world and have been reported in literature and also scientifically proven to have anti-asthmatic activity [2].

Respiratory disorders are one of the leading causes of mortality and morbidity affecting people of all ages and are major global health problem. The overall worldwide prevalence of bronchial asthma is approximately 4.5 percent [3]. Bronchial asthma is characterized by

chronic airway inflammation and hyper-responsiveness along with reversible airway obstruction. It is diagnosed by the history of respiratory symptoms like wheezing, shortness of breath, cough, chest tightness that varies over intensity and in time, accompanied with inconsistent expiratory airflow obstructions. Variations in expiratory airflow obstructions are triggered by different stimuli, including allergen exposure, exercise, viral-infection (cold) and respiratory airflow irritants like changes in weather, smoke and vehicle exhaust fumes. The features mentioned above for asthma generally continue even when lung function is normal or symptoms are absent, but may be controlled with treatment [4].

There are several agents that are used alone or in combination for the treatment of bronchial asthma. The functional abnormalities of the diseased respiratory system have been recognized by Unani system medicine. UNIM-352 is a formulation containing a combination of following constituents which include *Linum usitatissimum* L. (1g), *Trigonella-foenum-graecum* L. (2g), *Allium sativum* L. (3.5g), *Strychnos potatorum* L. (7g), *Caesalpinia bonducella* Fleming (1g) and *Pongamia glabra* Vent (1g), Honey (q.s.) in 20g of preparation [5,6]. The ingredients present in UNIM-352 are all well-known medicinal plants. This polyherbal traditional therapy has been proven efficacious in the management of asthmatic patients, and its cellular and molecular mechanisms of action has been proposed [6].

So, this study was designed to down size the number of ingredients in UNIM-352 and validate their effects in experimental models of asthma without affecting its efficacy and/or safety. Therefore, an attempt has been made to optimize UNIM-352 by comparing the efficacy and safety of the original formulation constituted of six (06) ingredients with the new optimized preparations (OP) with successive elimination of 1 to 3 ingredients, which were deemed less important in light of available scientific literature [7]. In this study effects, of the safety profile of optimized formulation of UNIM-352 was assessed as per AYUSH-OECD guidelines and compared with that of UNIM-352.

Materials and Methods

Animals

In this study, Wistar rats (180 - 250g) both males and females were used. Animals were kept in polyacrylic cages in standard controlled room temperature (22 ± 2°C) with natural light and dark cycle. Rats were fed with food pellets and water ad libitum. The protocol was approved by Institutional Animal Ethics Committee (IAEC/7/2015) following the guidelines of CPCSEA; Animal care was as per the guidelines of Indian National Science Academy (INSA), New Delhi.

Drugs and chemicals

UNIM-352 and various optimized preparations (OP) of UNIM-352 were used in our study. The formulations were prepared, standardized and supplied by Central Research Institute of Unani Medicine (CRIUM) Hyderabad, under the auspices of Central Council for Research in Unani Medicine (CCRUM) Ministry of AYUSH, Govt of India.

UNIM-352 classically contains six constituents viz. *Linum usitatissimum* L (1g), *Trigonella foenum-graecum* L (2g), *Allium sativum* L (3.5g), *Strychnos potatorum* L (7g), *Caesalpinia bonducella* Fleming (1g), *Pongamia glabra* Vent (1g) and Honey (q.s.) in 20g of preparation.

Optimized preparation-1 (OP-1) contained 3 ingredients: *Strychnos potatorum* L, *Trigonella-foenum-graecum* L, *Pongamia glabra* Vent and Honey.

Optimized preparation-2 (OP-2) contained 4 ingredients: *Strychnos potatorum* L, *Trigonella-foenum-graecum* L, *Pongamia glabra* Vent, *Allium sativum* L, and Honey.

Optimized preparation-3 (OP-3) contained 4 ingredients: *Strychnos potatorum* L, *Trigonella-foenum-graecum* L, *Pongamia glabra* Vent, *Linum usitatissimum* L, and Honey.

Optimized preparation-4 (OP-4) contained 4 ingredients: *Strychnos potatorum* L, *Trigonella-foenum-graecum* L, *Pongamia glabra* Vent, *Caesalpinia bonducella* Fleming, and Honey.

Chemicals

Ovalbumin, methacholine, and prednisolone were purchased from M/s Sigma-Aldrich, St Louis, MO, USA. Aluminium hydroxide, sodium chloride, hydrochloric acid, sodium hydroxide, propanol, perchloric acid, glacial acetic acid, formaldehyde, and other chemicals used in our study were purchased from Central Drug House (P) Ltd, New Delhi.

Immunization and challenge protocol

All rats in each group except normal group were immunized and challenged with OVA according to the procedure in previous study [8]. Briefly, rats were sensitized by intraperitoneal injection of OVA (10 mg per rat) emulsified with 10 µg of aluminium hydroxide in 0.5 ml of normal saline on the first day of the study. Fourteen days after sensitization, animals were challenged with 1 mg of ovalbumin.

Blood and bronchoalveolar lavage fluid collection (BAL) and cell counts

After 24 hrs of OVA challenge, all animals were anesthetized with ketamine dose 50 mg/kg and blood collected by cardiac puncture, the sample were centrifuged and Serum was separated and stored at -80°C. BAL fluid was retrieved by washing the lung through tracheal cannula with 0.9% sodium chloride solution and centrifuged at 1500 rpm at 4°C for 10 minutes. The supernatant recovered was stored at -80°C for the assay of various cytokine. The precipitated pellets were resuspended in 100 µl of normal saline. Eosinophil and Neutrophil counts in blood and BAL fluid were carried out using Neubauer chamber after Leishman staining [9].

Assessment of sub-acute oral toxicity of UNIM-352 and optimized formulations of UNIM-352 as per AYUSH-OECD guidelines (407)

The sub-acute toxicity (28 days) of UNIM-352 and optimized formulations of UNIM-352 was carried out as per AYUSH-OECD guidelines (407). The rats were selected randomly and conditioned for 7 days and divided into 6 groups of 6 wistar rats in each group were used at each dose level. The control group which is vehicle OVA sensitized and challenged and 5 test groups were administered 2000 mg/kg p.o daily of UNIM-352 or optimized formulation of UNIM-352. Animals were observed for a period of 28 days, for any abnormal sign and mortality. Weights of each animal were recorded at weekly intervals. At the end of 4^{th} week sensory reactivity (using photoactometer), motor reactivity and grip strength (using rotarod) were assessed. Following these test rats were anesthetized and the blood collection was done by cardiac puncture. Haematological parameters were determined using assay kits after sacrificing the animals. Tissue sample of lungs, Heart, brain of control and test groups were weighed and preserved in 10% formalin for further histopathological examination [10].

Statistical analysis

The data was expressed as mean ± S.E.M. Statistical analysis was done by using one-way analysis of variance (ANOVA) followed by post hoc Tukey`s test.

Results

Sub-acute oral toxicity of UNIM-352 and Optimized preparations of UNIM-352 as per AYUSH-OECD guidelines (407)

The sub-acute toxicity (28 days) of UNIM-352 and Optimized preparations of UNIM-352 was carried out as per AYUSH-OECD guidelines. The rats were selected randomly and then conditioned for 7 days. 6 wistar rats (3 males and 3 females) were administered with each preparation for 28 days at each dose level. The control group as administered vehicle (distilled water) and 5 test groups were administered 2000 mg/kg of UNIM-352 and four optimized preparations of UNIM-352. Animals were observed for a period of 28 days, for any abnormal sign and mortality. Weight of each animal was recorded at weekly intervals. Animals showed no toxic clinical signs throughout the dosing period of 28 days and exhibited body weight gain comparable to that of experimental control group.

Observations

Toxcity sign: Animals were free from any change in skin, fur, eyes, and mucous membrane, somatomotor activity, behavioural pattern (tremors, convulsions, salivation, diarrhoea, lethargy and sleep) sign of severe pain and distress and abnormality throughout the observation period of 28 days.

Functional observation: Sensory reactivity, motor reactivity and grip strength conducted in experimental sub-acute toxcity studies have not shown any abnormality and were same as seen in control group.

Mortality: All the animals from the control group and treatment grouped survived throughout the dosing period. The experimental control group which is OVA sensitized and challenged and 5 test groups were administered 2000 mg/kg of UNIM-352 and optimized preparations of UNIM-352 and there was no mortality in any of the treatment groups.

Body weight: Relative mean body weight of rats treated with UNIM-352 and optimized preparations of UNIM-352 showed comparable body weight gain throughout the dosing period of 28 days. These results were shown in table 1.

Body weight (gm)		Treatment (with 2000 mg/kg of each formulation)					
		Control	UNIM	OP-1	OP-2	OP-3	OP-4
Day -0	M	290 ± 10.0	270 ±15.3	263 ±13.3	273 ±14.5	270 ±10.0	300 ±11.5
	F	210 ± 5.8	227 ± 8.8	197 ± 3.3	180 ± 11.5	220 ±11.5	220 ±11.5
Day-7	M	298 ± 4.4	277 ± 16.7	270 ± 15.3	277 ± 14.9	272 ±10.3	301 ±11.0
	F	215 ± 8.7	234 ± 9.5	203 ± 1.5	183 ± 13.0	222 ±10.1	224 ±10.7
Day-14	M	310 ± 5.8	282 ± 16.5	274 ± 15.6	279 ± 16.3	275 ±10.0	307 ±11.7
	F	216 ± 8.7	234 ± 9.5	209 ± 0.7	186 ± 12.4	224 ±10.7	227 ±11.1
Day-21	M	313 ± 3.3	286 ± 16.4	275 ± 15.3	280 ± 16.1	277 ±11.1	308 ±11.6
	F	220 ± 8.4	238 ± 9.2	211 ± 0.7	189 ± 12.7	227 ±10.1	231 ±11.0
Day-28	M	315 ± 2.9	288 ± 15.9	277 ± 16.9	281 ± 16.7	278 ±11.3	310 ±11.5
	F	221 ± 8.7	239 ± 8.5	212 ± 1.5	191 ± 12.1	231 ±11.0	233 ±10.1

Table 1: *Effects of UNIM-352 and optimized preparations of UNIM-352 on mean body weight in sub-acute oral toxicity. All values are expressed as Mean ± SEM (n=6/group), UNIM-352, Optimized formulation (1-4); M: Male; F: Female.*

Gross pathological changes: The gross pathological examination of experimental control group, UNIM-352 and optimized preparation of UNIM-352 group of animals did not reveal any abnormalities. Macroscopic examination showed no remarkable change in the lung, liver, kidney, stomach, spleen, heart, brain and testis/ovaries of experimental animals.

Haematological investigation: UNIM-352 and optimized preparation treatment groups showed no significant change in the values of the different parameters viz. Haemoglobin, RBC count, total leukocyte count, differential leukocyte count, platelets, hematocrit, MCV (mean cell volume), MCH (mean cell hemoglobulin) and MCHC (mean cell hemoglobulin concentration) when compared to control group. These results are shown in table 2.

Haematological parameter		Treatment (with 2000 mg/kg of each formulation)					
		EC	UNIM	OP-1	OP-2	OP-3	OP-4
Hb (g/dl)	M	12.59 ± 0.53	14.76 ± 0.49	11.45 ± 0.17	12.23 ± 0.67	10.64 ± 1.19	10.98 ± 0.73
	F	13.23 ± 0.29	10.17 ± 0.45	11.87 ± 0.64	12.29 ± 0.29	13.49 ± 1.13	13.12 ± 0.76
TLC (10^3/cumm)	M	10.82 ± 2.8	12.76 ± 1.1	10.82 ± 0.7	11.66 ± 2.1	13.42 ± 1.2	10.90 ± 0.9
	F	5.38 ± 1.4	6.88 ± 1.2	6.08 ± 1.6	6.57 ± 1.2	4.72 ± 1.0	6.57 ± 1.1
Neutrophils (%)	M	18.8 ± 0.7	19.1 ± 1.2	20.2 ± 1.3	20.3 ± 1.6	21.5 ± 1.0	21.6 ± 0.8
	F	18.3 ± 0.6	20.3 ± 0.5	21.7 ± 1.1	22.9 ± 0.9	22.1 ± 1.1	21.4 ± 1.0
Lymphocytes (%)	M	81.4 ± 1.9	84.3 ± 1.7	83.8 ± 1.8	83.2 ± 1.5	81.8 ± 2.0	78.8 ± 4.2
	F	83.2 ± 0.8	83.5 ± 1.6	82.1 ± 3.3	79.2 ± 2.4	72.5 ± 1.3	79.8 ± 2.9
RBC (10^6/cumm)	M	10.0 ± 0.3	9.4 ± 0.2	9.3 ± 0.2	9.4 ± 0.2	9.5 ± 0.1	9.2 ± 0.2
	F	8.1 ± 0.3	8.1 ± 0.2	7.5 ± 0.2	8.4 ± 0.2	8.0 ± 0.1	8.2 ± 0.2
HCT (%)	M	53.3 ± 2.8	54.0 ± 1.6	54.1 ± 0.8	53.4 ± 2.0	54.3 ± 1.1	53.2 ± 1.2
	F	47.6 ± 0.9	47.1 ± 1.7	43.2 ± 0.4	50.1 ± 1.4	46.1 ± 1.2	45.8 ± 0.6
Basophils (%)	M	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	F	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Eosinophils (%)	M	2.4 ± 0.3	1.2 ± 0.2	1.9 ± 0.3	1.3 ± 0.1	2.6 ± 0.2	1.5 ± 0.3
	F	2.6 ± 0.4	2.4 ± 0.3	2.5 ± 1.2	2.7 ± 0.3	3.2 ± 0.8	4.1 ± 1.1
MCV (FL)	M	54.8 ± 1.3	56.6 ± 0.8	54.9 ± 1.1	55.5 ± 1.9	55.7 ± 0.9	56.6 ± 0.9
	F	59.1 ± 1.8	56.7 ± 1.7	58.1 ± 1.4	55.2 ± 1.2	57.7 ± 2.2	56.1 ± 1.4

Table 2: *Effects of UNIM-352 and optimized preparations of UNIM-352 on hematological parameters in sub-acute oral toxicity. All values are expressed in Mean ± SEM (n = 6/group), UNIM-352, Optimized formulation (1-4); MCV: Mean Corpuscular Volume; HCT: Hematocrit; M: Male; F: Female.*

Histopathology of various organs including heart, brain, lungs, using H and E staining was done after 28 days of treatment with UNIM-352 and optimized preparations of UNIM-352. The result did not show any remarkable change. However, in few male and female animals in both vehicle control and treatment groups there was presence of congested alveolar spaces in lung and congested red pulp in spleen which was within normal range. These results are summarized in figure 1-3.

Discussion

Natural goods, such as medicinal herbs, have been used to cure a variety of disorders for hundreds of years [3]. The assessment and evaluation of the hazardous properties of a natural product extract or compound is usually the first step in screening natural goods for pharmacological activity. Regardless of HS's pharmacological benefits, there is a scarcity of information about the toxic consequences of this well-known herb. As a result, the current investigation was carried out in rats to assess and focus on the sub-acute toxicity of UNIM-352, optimized preparations of UNIM-352. The results of repeated dose toxicity testing reveal toxic effects, target organ identification, and impacts on animal physiology, haematology, metabolic profile, and histology. Regulatory bodies require these tests to characterise the toxicological potential of any chemical [11].

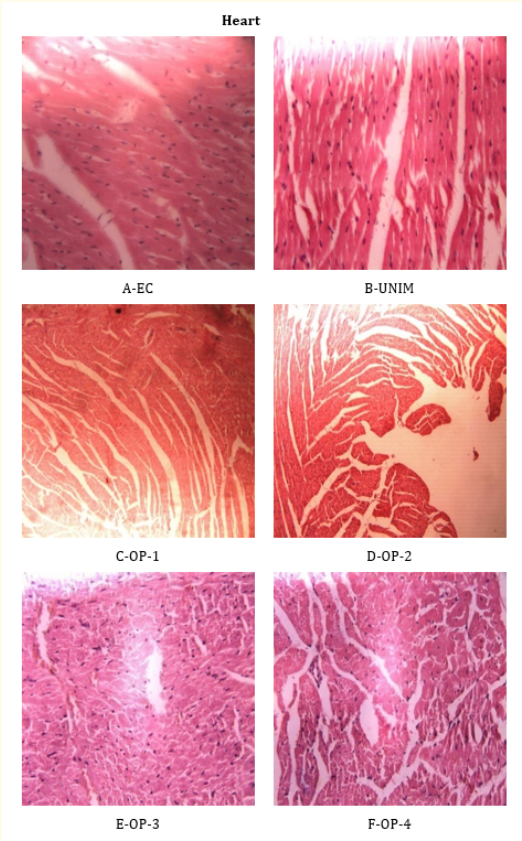

Figure 1: *Histopathological analysis of heart sections stained with Hematoxylin and Eosin 24 hour after last sub-acute oral dosing in Wi-star rats; A- Experimental control, B- UNIM-352, C- Optimized preparation-1, D- Optimized preparation-2, E- Optimized preparation-3, F- Optimized preparation-4.*

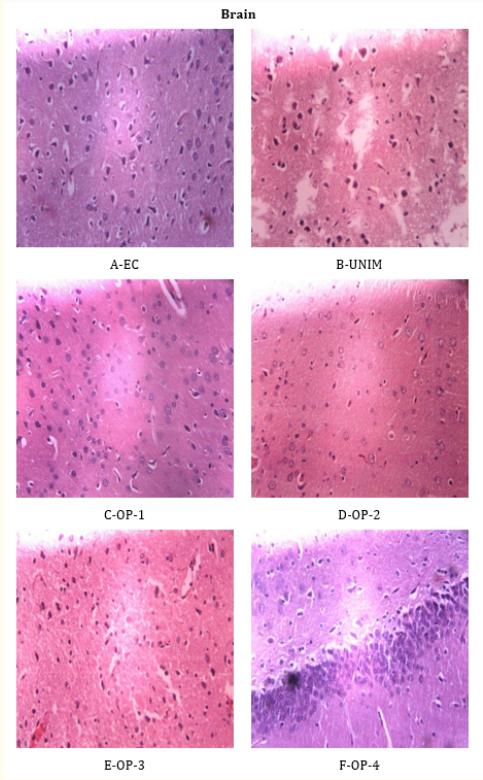

Figure 2: *Histopathological analysis of brain sections stained with Hematoxylin and Eosin 24 hour after last sub-acute oral dosing in wistar rats; A- Experimental control, B- UNIM-352, C- Optimized preparation-1, D- Optimized preparation-2, E- Optimized preparation-3, F- Optimized preparation-4.*

Toxicity evaluation of the compound provides information regarding possible health hazard that can occur from single or repeated exposure on different biological. Hence, it is important to identify the potential harmful effects of a compound of therapeutic use. According to regulatory guidelines with traditional products, sub-acute toxicity studies of the various optimized preparations were assessed [10]. The experimental animals were observed for various parameters to evaluate sub-acute oral toxicity of UNIM-352, optimized preparations of UNIM-352 were administered at dose of 2000 mg/kg to wistar rats for 28 days by oral gavage and observed for any mortality. As there was no immediate mortality the animals were observed for period of 28 days and found that all the animals were free from any change in eyes, fur, skin, mucous membrane, somatomotor activity and behavioral pattern (tremors, convulsions, salivation, diarrhea, sleep and lethargy), sign of severe pain and distress and abnormality throughout the observation period of 28 days. The functional observation including sensory reactivity, motor reactivity and grip strength conducted in experimental rats did not reveal any abnormality. Relative mean body weight in UNIM-352 and optimized preparations of UNIM-352 showed comparable increase in the mean body weight as observed in the vehicle control group. Gross pathological examination of various organs (stomach, kidney, lungs, heart, brain, testis and ovaries) did not reveal any abnormal sign of macroscopic examination, suggesting safety of our optimized preparations.

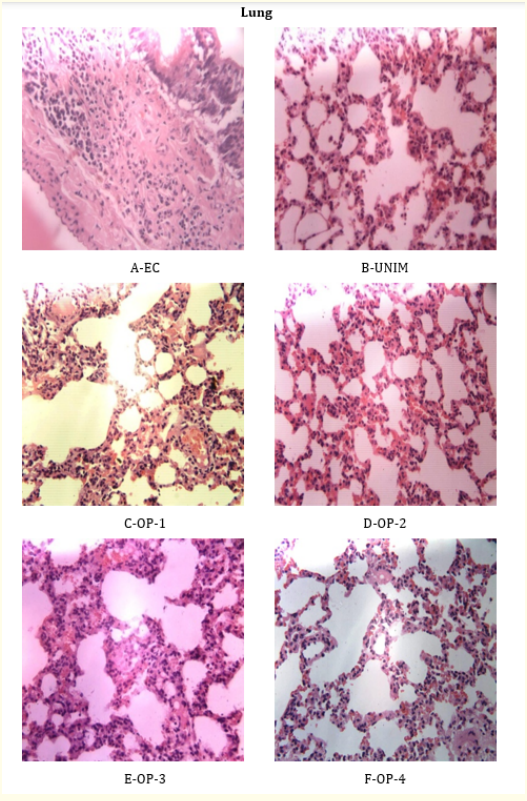

Figure 3: *Histopathological analysis of lung sections stained with Hematoxylin and Eosin 24 hour after last sub-acute oral dosing in Wistar rats; A- Experimental control, B- UNIM-352, C- Optimized preparation-1, D- Optimized preparation-2, E- Optimized preparation-3, F- Optimized preparation-4.*

Liver and kidney function tests are the most important parameters for evaluating the toxicity of the optimized formulations. Liver function test is used for the assessment of kinetic activity of the important enzyme which includes Aspartate-S- Transferase (AST), Alanine-L-Transferase (ALT) and Alkaline Phosphorylase (ALP) and increase in the levels of these enzymes is a sign of liver injury [12]. In our study, we found no significant difference in the serum levels of ALT, AST and ALP in optimized preparations vs vehicle treated control group and the values were within biological range. There was no significant change in the serum concentration of albumin and bilirubin after treatment with different optimized preparations and found that our formulations are safe in liver function test. Serum concentration of urea and creatinine are the most important parameter of kidney function test [13]. Administration of optimized formulations of UNIM-352 did not affect the levels of urea and creatinine and are shown to be completely safe in kidney.

Hematopoietic system which includes blood cells and their functions and thus is more susceptible to toxic compounds [14]. Sub-acute administration (for 28 days) of optimized preparations of UNIM-352 did not cause any significant changes in the hematological parameters of both male and female rats as compared vehicle control group. This shows that nontoxic effects of optimized formulation of UNIM-352 to hematopoietic system.

Conclusion

The safety of UNIM-352 and optimized preparations of UNIM-352 were evaluated by sub-acute oral toxicity studies in rats as per OECD guidelines. The sub-acute toxicity study of optimized preparations of UNIM-352 showed no toxicity sign with body weight gain compared

to that in experimental control group of rats. Mean organ body weight, hematological and biochemical parameter in treated groups were within normal biological range. Histopathological examination of various organs showed no abnormal structural abnormalities. The results of sub-acute toxicity studies showed that optimized preparations of UNIM-352 were safe upto the dose of 2000 mg/kg.

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