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RESEARCH ARTICLE

Safety and toxicological evaluation of Aflapin®: A novel Boswellia-derived anti-inflammatory product

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Abstract

Boswellia serrata gum resin has been used for treatment of various ailments in different cultures for thousands of years. Aflapin® is a novel synergistic composition derived from *B. serrata* gum resin (Indian Patent Application No. 2229/CHE/2008). Aflapin is significantly better as an anti-inflammatory agent compared to the Boswellia extracts presently available in the market. To assess the safety of Aflapin, a battery of acute and sub-acute toxicity studies were conducted in various animal models according to the OECD test guidelines. The acute oral LD50 of Aflapin was greater than 5000 mg/kg in female Sprague Dawley (SD) rats. Acute dermal LD50 of Aflapin was greater than 2000 mg/kg in SD rats. A primary dermal irritation study conducted using New Zealand White rabbits indicated that Aflapin is non-irritating to skin. Aflapin caused minimal ocular irritation in a primary eye irritation test conducted on New Zealand Albino rabbits. A repeat dose 28-day sub-acute oral toxicity study in SD rats demonstrated no significant signs of toxicity. Various evaluations including hematology, clinical chemistry, gross necropsy, and histopathology did not show any significant adverse changes. The NOAEL of Aflapin was found to be greater than 2500 mg/kg body weight. These studies demonstrate broad spectrum safety of Aflapin in animal models.

Keywords: *Acute oral toxicity; acute dermal toxicity; Aflapin; anti-inflammatory;* Boswellia serrata*; New Zealand albino rabbit; primary eye irritation; primary skin irritation; Sprague-Dawley rat; sub-acute toxicity*

Introduction

Ayurveda is one of the oldest (more than 5000 years) systems of Medicine originated from Vedic culture of India to provide healthy life to mankind. In Sanscrit, '*ayus*' means 'life' and '*ved*' signifies 'knowledge or science' (Dev 1999). *Boswellia serrata* (frankincense), also known as salai guggul, is one of the most ancient and respected herbs in Ayurveda (Sastri 1962; Aman and Balu 2009). *B. serrata* is a moderate-to-large branching tree found in India, Northern Africa, and the Middle East. *B. serrata* gum resin has been used for treatment of various aliments in different cultures for thousands of years (Ethan et al. 2004; Ammon 2008; Guptha et al. 1998).

Aflapin® is a novel synergistic composition comprising *Boswellia serrata* extract selectively enriched in AKBA (Indian Patent Application No. 2229/CHE/2008). Aflapin® possesses superior efficacy as an anti-inflammatory and anti-osteoarthritis agent, and exhibits better bioavailability compared to other *B. serrata* extracts commercially available in the market (being communicated as a separate manuscript). The superior efficacy of Aflapin® has been a motivation to the authors to assess its broad spectrum safety in appropriate animal models.

Materials and methods

Chemicals

Aflapin was obtained from Laila Nutraceuticals (Vijayawada, India), and was used for all studies reported here. Aflapin material used in these studies was tested for heavy metals, microbes, pesticide residues, and aflatoxins, and the contamination levels were found to be within the acceptable limits as per USP guidelines. Biochemical reagents and hematology cell pack were obtained from Human GmbH 65205 (Wiesbaden, Germany). Unless otherwise stated, all other chemicals were obtained from Sigma Chemical Company (St Louis, MO).

Housing of experimental animals

Animals study protocols were approved by the Institutional Animal Ethics Committee (IAEC). All the studies were

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performed at Laila Impex R&D Center in compliance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and OECD. Sprague Dawley (SD) rats, New Zealand White (NZ) rabbits, and standard animal diets were obtained from the National Institute of Nutrition (Hyderabad, India). Animals were provided with standard diet and charcoal filtered and UV exposed water ad libitum. Rats were housed in 15″×9″×7″ polypropylene cages with stainless steel grill floors with sterilized rice husk used as bedding. Rabbits were housed in 24″×24″×20″ suspended stainless steel cages. Prior to dose initiation the rats and rabbits were acclimated to laboratory conditions for at least 7 and 21 days, respectively. The animal room was maintained at a controlled temperature (24–26°C), humidity (45–70%), and light/dark cycle (12 h/12 h).

Acute oral toxicity study in female SD rats

Acute oral toxicity study using up-and-down procedure was conducted in compliance with the principles of good laboratory practice as set forth in the OECD test guidelines 425.

A limit test was performed using three female SD rats (12 weeks old; body weight 210–230 g at study initiation) following the methodology described in the authors' earlier publications (Lalithakumari et al. 2006; Krishnaraju et al. 2009; 2010). Briefly, Aflapin was administered at a single dose of 5000 mg/kg. Dose formulation of Aflapin was prepared prior to administration. Aflapin was suspended in 0.5% CMC in water to obtain a final concentration of 500 mg/ml (50% w/v) to allow a final dosage volume of 10 ml/kg body weight. Aflapin was administered to female SD rats by oral gavage using a suitably graduated syringe and a stainless steel (18 gauge) intubation cannula. All animals were sacrificed at the end of the observation period and the key organs and tissues were subjected to a complete macroscopic examination.

Acute dermal toxicity study in SD rats

An acute dermal toxicity test was conducted in rats to determine the potential of Aflapin to cause toxicity from a single topical application. This study was conducted following the methodology described in the authors' earlier publications (Lalithakumari et al. 2006; Krishnaraju et al. 2009; 2010). Briefly, five male and five female (nulliparous and non-pregnant) rats of 8–10 weeks age having 190–210 g and 200–250 g body weight, respectively, were used in this experiment.

Individual body weights of the animals were recorded prior to test substance application (initial) and again on days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice.

The animals were observed for mortality, signs of gross toxicity and behavioral changes after sample application, and at least once daily thereafter for 14 days. All rats were euthanized via CO_2 inhalation on day 14. Gross necropsies were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined.

Primary skin irritation study in male and female rabbits

A primary skin irritation test was conducted on young adult New Zealand albino rabbits to determine the potential for Aflapin to cause irritation after a single topical application, as per the methodology described in the authors' earlier publications (Lalithakumari et al. 2006; Krishnaraju et al. 2009; 2010). One day before application, the hair from the dorsal trunk area of the rabbits was clipped using Oster® #A5-small animal clipper. Prior to sample application, the clipped skin was checked for abnormalities including signs of skin irritation. Three healthy animals (two males and one nulliparous and non-pregnant female) without pre-existing skin irritation were selected for study.

Aflapin was moistened with distilled water to achieve a dry paste by preparing a 75% w/w mixture. Five hundred milligrams of the Aflapin test mixture (670 mg of wet mixture) was placed on a 4-ply gauze pad and applied to 6 cm^2 intact dose site on each animal. The pad and entire trunk of each animal were then secured with semi-occlusive 3-inch tape to avoid dislocation of the pad. Elizabethan collars were placed on each rabbit and they were returned to their designated cages. After 4 h of exposure to Aflapin, the pads and collars were removed and the test sites were gently cleansed of any residual test substance.

Individual evaluation of test sites was scored according to the dermal irritation scoring system (Draize 1965). The scores were recorded at 1, 24, 48, and 72 h after removal of Aflapin. The classification of irritancy was obtained by calculating the Primary Dermal Irritation Index (PDII) and classified according to the EPA guidelines (Health Effects Test Guidelines, Acute dermal Irritation (OPPTS 870.2500, 1998)).

Primary eye irritation study in male and female rabbits

The objective was to determine the potential of Aflapin to cause irritation from a single ocular instillation. This study was conducted in accordance with OECD Test Guidelines 405.

Three healthy young adult New Zealand albino rabbits (two males and one female (nulliparous and non-pregnant)) were used for the study. The route of Aflapin administration was direct conjunctival instillation, a standard procedure for assessment of local ocular irritation. One tenth of a gram of Aflapin was instilled into the conjunctival sac of the right (test) eye of each rabbit by gently pulling the lower lid away from the eyeball. The upper and lower lids were then gently held together for ~ 1 s before releasing to minimize loss of the test substance. The left (control) eye of each animal remained untreated.

Following treatment, ocular irritation was evaluated macroscopically using a high-intensity white light (Mag Lite) at 1, 24, 48, and 72 h post-instillation. Individual eye irritation scores were recorded for each animal. Ocular lesions were scored according to the scale for scoring eye lesions (Draize et al. 1944). The average score for all rabbits at each scoring point was calculated to aid in data interpretation. Classification of eye irritation scores obtained at 1, 24, 48, and 72 h was done following the primary eye irritation scores system (Kay and Calandra 1962). The rabbits were also

monitored at least once daily for mortality and signs of gross toxicity and behavioral changes during the test period.

Sub-acute (28-day) repeated dose oral toxicity studies

The objective of the repeat dose 28 day oral toxicity study was to evaluate the safety of Aflapin in male and female SD rats conducted according to OECD test guidelines 407. Male and female SD rats (males weighing 175–250 g; females weighing 165–200 g) were used for this study. The animals were given free access to standard rodent diet during acclimatization and treatment phases of the experiment.

The study was conducted following the methodology described in the authors' earlier publications (Krishnaraju et al. 2009; 2010). Briefly, the animals were supplemented with daily oral doses of either 0, 50 (400 mg human equivalency dose (HED)) or 250 mg/kg (2000 mg HED) or 2500 mg/ kg (20,000 mg HED) of Aflapin for 28 consecutive days.

Terminal necropsy of all animals was carried out and the organ weights (adrenals, heart, liver, sex organs, kidney, brain, spleen, and thymus) were recorded. The organ weights were normalized to percentage body weight of respective rat. Subsequently, target organs including adrenal glands, brain, epididymides, esophagus, eyes, heart, intestine, kidney, liver, lymph nodes, lungs, mammary glands, ovaries or testes, pancreas, pituitary, prostate, salivary glands, seminal vesicles, skin, spleen, stomach, thymus gland, thyroid gland, trachea, and urinary bladder were collected. The organs were weighed and fixed in phosphate buffered 10% formalin for histopathology examination. The tissues were processed, embedded in paraffin wax, cut into thin sections, and stained with haematoxylin and eosin. The stained sections were examined under a light microscope at either 10× or 20× objective of Nikon T-100FS microscope (Nikon Corporation, Tokyo, Japan).

Statistical analysis

All data were expressed as the mean ± SD. One way analysis of variance (ANOVA) analysis was performed to detect further difference between groups. Values of $p < 0.05$ were considered significant.

Results

Acute oral toxicity study in female rats

In this study, single oral administration of Aflapin® was given to female SD rats to assess its acute toxicity, as a limit test using up and down procedure. At the limit dose of 5000 mg/ kg body weight, Aflapin® did not cause mortality in any of the three animals tested and did not show any signs of toxicity in rats following dosing and during the observation period of 14 days thereafter. The body weight gain of treated rats was normal. No gross pathological alterations were encountered in any of the rats, as evident at terminal necropsy. Based on these results and under the conditions of this study, the median lethal dose (LD50) of Aflapin after single oral administration in female SD rats was expected to be more than the limit dose level of 5000 mg/kg body weight.

Acute dermal toxicity study in SD rats

An acute dermal toxicity test was conducted using male and female SD rats to determine the potential of Aflapin to cause toxicity from a single topical application. Under the conditions of this study, the acute dermal LD50 of Aflapin was found to be greater than 2000 mg/kg body weight in male and female rats. All animals survived, gained weight, and appeared active and healthy. There were no signs of gross toxicity, dermal irritation, or adverse pharmacological effects or abnormal behavior. No gross abnormalities were observed for any of the animals upon terminal necropsy.

Primary skin irritation study in male and female rabbits

The objective of this study was to determine the potential of Aflapin to cause dermal irritation from a single topical application to the skin of NZ rabbits. A summary of primary dermal irritation scores is presented in Table 1. All animals were free from dermal irritation till 48 h. Under the conditions of this study, the primary dermal irritation index for Aflapin is '0'. Thus, Aflapin can be classified as non-irritating to the skin. All animals appeared active and healthy with no other signs of gross toxicity or adverse pharmacological effects or abnormal behavior.

Primary eye irritation study in male and female rabbits

A primary eye irritation test was conducted in NZ albino rabbits to determine the potential for Aflapin to cause irritation from a single instillation via the ocular route. No corneal opacity or iritis was observed in any treated eye during the study. One hour following test substance instillation, all treated eyes exhibited conjunctivitis, and the same was subsided by 24 h. Thereafter, all animals remained free of ocular irritation up to 72 h. Apart from the eye irritation observed, all animals appeared active and healthy. There were no other signs of gross toxicity or adverse pharmacological effects or abnormal behavior. Under the conditions of this study, the maximum mean total score (MMTS) of Aflapin is 4.0, thus classifying Aflapin to be minimally-irritating to the eye (Table 2).

Sub-acute oral toxicity study in SD rats

Effect on food consumption, body weights and organ weights

The weekly food consumption data is shown in Table 3. Male rats consumed ~ 20–30% more feed when compared to the female rats. No significant reduction in food consumption

Table 1. Primary dermal irritation scores in male and female New Zealand albino rabbits.*

Time post-instillation (h)	Incidence of dermal irritation		Total primary dermal irritation (PDI)**	Primary dermal irritation index (PDII)
	Erythema	Edema	Mean score	
1	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	
48	0.0	0.0	0.0	
72	0.0	0.0	0.0	

* Average values ($n = 3$).

** Primary dermal irritation = average erythema + average edema.

was observed in any of the treatment groups compared to the respective control group. The changes in body weights following supplementation of Aflapin to the male and female rats are presented in Table 4. Although the initial body weights of both male and female rats were very close, the male rats gained more weight over the period of 28 days as compared to the female rats. No significant reduction in body weight was observed in either female or male animals compared to respective control groups.

Selected organs, including adrenal glands, brain, heart, kidneys, liver, prostate and seminal vesicles, spleen, testes, and thymus in male rats, and adrenal glands, brain, heart, kidneys, liver, ovaries, spleen, thymus, and uterus in female rats were weighed and noted as such, and expressed as a percentage of body weight after 28 days of treatment, as summarized in Table 5. No significant differences were observed in organ weights of treated male and female animals when compared to respective controls.

Effect on hematology and clinical chemistry

Tables 6 and 7 demonstrate the hematology and clinical chemistry data, respectively, for male and female SD rats, which were treated with 0.0 mg/kg, 50 mg/kg, 250 mg/kg, and 2500 mg/kg Aflapin for 28 days. White blood cells (WBC), red blood cells (RBC), hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular concentration, platelet count, lymphocyte, monocyte, neutrophil, eosinophils, basophils, total serum protein, total albumin, alkaline phosphatase, urea, creatinine, SGOT, SGPT, cholesterol, triglycerides, total bilirubin, glucose, sodium, and potassium were determined in each animal. Significant reduction in total cholesterol was observed in the female animals compared to control. The low dose treatment resulted in significant ($p < 0.05$) reduction and the medium and high dose treatment resulted in highly significant ($p < 0.01$) reduction in serum total cholesterol levels. There were no other significant changes observed in any of the treatment groups as compared to the respective control groups.

Table 2. Incidence, severity, and reversibility of ocular irritation in New Zealand albino rabbits after exposure to Aflapin.

Time post-instillation (h)	Incidence of ocular irritation: Corneal opacity	Iris	Conjunctivitis	Severity of irritation MMT* score
1	0/3	0/3	3/3	4.0
24	0/3	0/3	0/0	0.0
48	0/3	0/3	0/0	0.0
72	0/3	0/3	0/0	0.0

* Maximum mean total score.

Table 3. Effect of Aflapin on the food intake of male and female Sprague-Dawley rats 28 days after treatment.

	0 mg/kg Aflapin		50 mg/kg Aflapin		250 mg/kg Aflapin		2500 mg/kg Aflapin	
Days	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Male								
1–4	19.55	1.01	19.35	0.99	19.00	1.47	17.45	3.05
5–8	18.05	3.06	19.45	0.96	18.60	1.95	17.10	2.59
9–12	19.70	0.54	19.70	0.33	18.80	2.27	18.65	1.43
13–16	19.25	1.12	18.90	0.68	18.65	1.63	18.70	1.87
17–20	18.00	1.63	18.05	1.30	18.25	1.96	18.15	1.07
21–24	18.00	2.01	17.70	1.39	17.45	1.79	17.15	1.61
25–28	17.80	1.81	17.70	1.11	17.30	1.92	17.10	1.67
Female								
1–4	12.85	0.55	13.40	0.84	15.10	1.33	13.05	1.80
5–8	12.90	1.13	13.50	1.48	14.25	1.05	13.90	2.10
9–12	14.20	0.60	14.05	1.36	14.40	1.27	13.70	2.19
13–16	11.85	1.14	12.70	0.74	13.45	0.65	12.25	2.37
17–20	12.30	0.65	12.60	0.80	14.50	1.16	13.55	2.57
21–24	11.70	0.48	12.85	1.32	13.50	0.77	14.80	1.45
25–28	12.35	1.13	12.40	0.84	13.10	1.33	13.20	2.11

Sprague-Dawley rats were individually treated daily with an oral dose of Aflapin at 0 mg/kg (control), 50 mg/kg, 250 mg/kg, and 2500 mg/kg for 28 days. Animals were sacrificed after 28 days of treatment. Each value represents the mean ± SD, $n = 5$.

Histopathology

There were no adverse histological findings clearly attributable to the treatment. The histological changes noted were sporadic or common background findings and not likely to be related to the treatment. Mild-to-moderate congestion was observed in various organs of both the treatment and control group of animals. There was no dose relation or difference observed in any histological findings when compared to the control group.

Overall, these data demonstrate the broad spectrum subacute safety of Aflapin, at the dosage up to 2500 mg/kg per day in male and female Sprague-Dawley rats under the conditions of the study.

Discussion

A battery of toxicity studies focused to determine and demonstrate the broad spectrum safety of Aflapin, a novel synergestic Boswellia derived composition. The *B. serrata* extracts have been known to be safe for human consumption for thousands

Table 4. Effect of Aflapin on the body weights of male and female Sprague-Dawley rats after 28 days of treatment.

Day	0 mg/kg Aflapin	50 mg/kg Aflapin	250 mg/kg Aflapin	2500 mg/kg Aflapin
Male				
0	219.6 ± 8.29	222.8 ± 21.81	217.8 ± 16.63	221.8 ± 20.39
7	259.0 ± 19.40	277.0 ± 19.00	262.6 ± 27.38	252.4 ± 22.04
14	294.4 ± 14.96	307.8 ± 18.20	293.6 ± 33.40	285.6 ± 25.32
21	322.2 ± 13.92	335.0 ± 17.29	315.6 ± 27.85	310.2 ± 28.39
28	322.8 ± 14.62	329.6 ± 16.55	314.4 ± 31.79	294.2 ± 25.40
Female				
0	185.6 ± 5.18	178.2 ± 10.47	190.6 ± 7.70	186.2 ± 6.38
7	208.4 ± 9.56	201.0 ± 12.31	217.8 ± 12.42	210.4 ± 15.96
14	221.2 ± 7.33	217.6 ± 14.77	235.2 ± 14.02	214.8 ± 16.89
21	231.0 ± 5.43	222.0 ± 11.14	245.6 ± 14.01	222.2 ± 15.37
28	216.0 ± 2.55	214.4 ± 13.37	231.0 ± 13.19	208.4 ± 9.71

Sprague-Dawley rats were individually treated daily with an oral dose of Aflapin at 0 mg/kg (control), 50 mg/kg, 250 mg/kg, and 2500 mg/kg for 28 days. Animals were sacrificed after 28 days of treatment. Each value represents the mean ± SD, $n = 5$.

Table 5. Effects of Aflapin on body weight and vital organs of male and female Sprague-Dawley rats after 28 days of treatment.

Organs	0 mg/kg Aflapin	50 mg/kg Aflapin	250 mg/kg Aflapin	2,500 mg/kg Aflapin
Male				
Body weight	322.8 ± 14.62	329.6 ± 16.55	314.4 ± 31.79	294.2 ± 25.40
Adrenal glands (pair)	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
% Body weight	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00
Brain	1.92 ± 0.02	1.91 ± 0.10	1.84 ± 0.08	1.88 ± 0.06
% Body weight	0.59 ± 0.03	0.57 ± 0.02	0.58 ± 0.01	0.64 ± 0.04
Heart	1.03 ± 0.06	1.07 ± 0.06	1.01 ± 0.01	0.95 ± 0.07
% Body weight	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.02	0.32 ± 0.02
Kidney	2.51 ± 0.28	2.6 ± 0.17	2.42 ± 0.29	2.56 ± 0.39
% Body weight	0.77 ± 0.06	0.8 ± 0.03	0.76 ± 0.03	0.87 ± 0.06
Liver	14.01 ± 2.07	13.7 ± 0.77	13.86 ± 1.41	15.73 ± 1.86
% Body weight	4.33 ± 0.57	4.1 ± 0.28	4.45 ± 0.72	5.33 ± 0.18
Seminal vesicles	1.15 ± 0.33	1.2 ± 0.23	1.12 ± 0.37	1.10 ± 0.23
% Body weight	0.35 ± 0.10	0.4 ± 0.08	0.36 ± 0.12	0.37 ± 0.07
Spleen	0.46 ± 0.06	0.5 ± 0.03	0.45 ± 0.09	0.45 ± 0.09
% Body weight	0.14 ± 0.02	0.1 ± 0.01	0.16 ± 0.03	0.15 ± 0.02
Thymus	0.44 ± 0.06	0.5 ± 0.07	0.46 ± 0.08	0.45 ± 0.08
% Body weight	0.14 ± 0.02	0.1 ± 0.02	0.15 ± 0.02	0.15 ± 0.02
Testes	2.84 ± 0.20	2.8 ± 0.03	2.82 ± 0.19	2.86 ± 0.15
% Body weight	0.88 ± 0.04	0.85 ± 0.05	0.90 ± 0.07	0.97 ± 0.18
Female				
Body weight	216 ± 2.55	214.4 ± 13.37	231 ± 13.19	208.4 ± 9.71
Adrenal glands (pair)	0.05 ± 0.01	0.07 ± 0.00	0.06 ± 0.01	0.05 ± 0.01
% Body weight	0.02 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.01
Brain	1.78 ± 0.09	1.78 ± 0.15	1.78 ± 0.10	1.52 ± 0.53
% Body weight	0.82 ± 0.05	0.83 ± 0.05	0.77 ± 0.05	0.73 ± 0.25
Heart	0.74 ± 0.03	0.76 ± 0.05	0.82 ± 0.05	0.72 ± 0.03
% Body weight	0.34 ± 0.01	0.36 ± 0.01	0.35 ± 0.01	0.35 ± 0.02
Kidney	1.82 ± 0.06	1.93 ± 0.08	2.08 ± 0.15	1.92 ± 0.16
% Body weight	0.85 ± 0.03	0.90 ± 0.02	0.9 ± 0.06	0.92 ± 0.07
Liver	9.93 ± 1.07	11.53 ± 1.13	12.32 ± 1.25	12.4 ± 0.30
% Body weight	4.6 ± 0.54	5.37 ± 0.33	5.34 ± 0.56	5.96 ± 0.21
Ovaries	0.12 ± 0.01	0.10 ± 0.01	0.13 ± 0.02	0.09 ± 0.03
% Body weight	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Spleen	0.44 ± 0.05	0.43 ± 0.04	0.38 ± 0.05	0.38 ± 0.05
% Body weight	0.20 ± 0.02	0.20 ± 0.01	0.19 ± 0.01	0.18 ± 0.02
Thymus	0.39 ± 0.13	0.42 ± 0.01	0.44 ± 0.03	0.33 ± 0.03
% Body weight	0.18 ± 0.06	0.19 ± 0.02	0.19 ± 0.01	0.16 ± 0.02
Uterus	0.47 ± 0.14	0.37 ± 0.07	0.53 ± 0.12	0.43 ± 0.14
% Body weight	0.22 ± 0.07	0.17 ± 0.03	0.23 ± 0.06	0.20 ± 0.06

Sprague-Dawley rats were individually treated daily with an oral dose of Aflapin at 0 mg/kg (control), 50 mg/kg, 250 mg/kg, and 2500 mg/kg for 28 days. Animals were sacrificed after 28 days of treatment. Each value represents the mean ± SD of five animals.

of years. The safety profiles of various other Boswellia products were tested earlier in different animal models (Singh and Atal 1986; Lalithakumari et al. 2006; Sharma et al. 2009). Clinical efficacy and tolerability of Boswellia products were tested in earlier clinical studies (Sontakke et al. 2007; Sengupta et al. 2008).

In the present acute oral toxicity study, there were no mortalities or gross pathological changes observed. In addition, the body weights were not significantly changed in Aflapin-treated rats. These results demonstrate that the LD50 of Aflapin is greater than 5000 mg/kg body weight, when administered as a single oral dose to female SD rats. In the acute dermal toxicity study, there was no mortality, no signs of gross toxicity, dermal irritation, adverse pharmacological effects, or abnormal behavior. No adverse macroscopic findings related to Aflapin treatment were found in any of the organs at the terminal necropsy. The acute dermal LD50 of Aflapin was found to be greater than 2000 mg/kg. In the primary dermal irritation study, the irritation index of Aflapin is '0.' Hence, Aflapin is classified as non-irritating to the skin. In the primary eye irritation study conducted in NZ rabbits, no corneal opacity or iritis was observed in any of the Aflapin treated eyes. The maximum mean total score (MMTS) for Aflapin is 4.0 at 1 h and completely subsided by 24 h. Hence, Aflapin is classified to be minimally irritating to the eye.

The sub-acute oral toxicity study was conducted to determine the toxic effects and potential target organs of toxicity to Aflapin following administration for 28

Table 6. Hematology results of control, low dose (50 mg/kg), medium dose (250 mg/kg), and high dose (2500 mg/kg) treated male and female Sprague-Dawley rats.

Days		WBC ($\times 10^3$/mm^3)	RBC ($\times 10^3$/mm^3)	Hemo globin (g/dL)	Hemato crit(%)	MCV(Fl)	MCHC(Pg)	MCC(%)	Platelet count (Million/ml)	Nutro phils(%)	Lympho cyte(%)	Eosino phil(%)	Mono cyte(%)	Baso phil(%)
Male														
Control														
28	Mean	11.25	8.244	13.92	50.16	61	27.62	16.72	826.4	16.60	77	1	5.4	0
	SD	1.47	0.306	0.409	1.544	1.414	0.576	0.559	124.9	4.88	6.08	0.71	2.07	0.00
Low dose														
28	Mean	10.88	8.458	14.44	52.4	62.2	27.5	17.04	802.2	19.60	73.60	1.20	5.60	0.00
	SD	1.087	0.254	0.434	1.673	1.483	0.339	0.391	80.49	10.81	12.10	1.10	2.30	0.00
Medium dose														
28	Mean	13.02	7.956	13.34	48.42	61	27.6	16.76	838.4	18.00	72.80	1.20	8.00	0.00
	SD	2.637	0.346	0.73	4.079	2.739	0.949	0.336	151.9	2.12	3.35	0.45	2.55	0.00
High dose														
28	Mean	11.32	8.408	14.04	50.86	60.4	27.58	16.68	955	13.40	80.60	0.60	5.40	0.00
	SD	1.213	0.173	0.241	1.083	0.548	0.179	0.164	64.4	5.59	6.84	0.89	1.82	0.00
Female														
Control														
28	Mean	8.904	8.132	14.04	51.84	64	27.06	17.26	867.6	14.80	79.40	0.60	5.20	0.00
	SD	1.567	0.332	0.404	1.806	1.581	0.391	0.518	203.5	4.49	7.47	0.89	2.28	0.00
Low dose														
28	Mean	10.66	8.318	14.32	52.52	63.2	27.26	17.24	755	13.60	79.00	1.00	6.40	0.00
	SD	2.421	0.422	0.438	1.553	1.483	0.279	0.416	82.01	2.07	3.74	1.22	2.61	0.00
Medium dose														
28	Mean	8.982	8.17	13.98	52.22	64	26.76	17.12	732	15.40	77.00	0.60	7.00	0.00
	SD	1.455	0.514	0.826	3.484	1.225	0.297	0.402	60.82	1.67	2.74	0.89	1.00	0.00
High dose														
28	Mean	9.528	8.456	14.06	51.74	61.2	27.2	16.64	980.6	13.40	78.00	1.20	7.40	0.00
	SD	1.311	0.268	0.397	1.795	1.643	0.604	0.207	68.44	1.67	2.74	0.45	1.14	0.00

Table 7. Clinical chemistry results of control, low dose (50 mg/kg), medium dose (250 mg/kg), high dose (2500 mg/kg) treated male and female Sprague-Dawley rats.

Days		ALB(g/dL)	ALP(mg/dl)	SGPT(IU/L)	SGOT (IU/L)	Bil-T (mg/dL)	Cal(mg/dL)	Chol (mg/dL)	Creat- (mg/dL)	Glu(mg/dL)	T.P.(g/dL)	TRG (mg/dL)	Urea (mg/dL)	Sod (Mmol/L)	Pot(mg/dL)
Male															
Control															
28	Mean	3.37	108.40	88.60	96.00	0.28	8.73	89.40	0.27	114.00	6.50	75.60	42.87	120.40	3.92
	SD	0.39	8.44	11.97	15.59	0.14	0.48	7.64	0.08	7.45	0.36	5.94	2.26	12.82	0.33
Low dose															
28	Mean	3.53	114.80	85.40	93.40	0.22	8.86	88.20	0.29	108.40	7.06	80.00	43.37	128.40	4.28
	SD	0.29	4.27	9.56	8.44	0.04	0.22	6.87	0.12	7.57	0.39	1.00	2.84	2.70	0.40
Medium dose															
28	Mean	3.64	118.00	81.80	89.20	0.19	9.11	84.80	0.28	117.80	6.64	77.60	43.78	127.60	3.80
	SD	0.58	3.39	8.38	9.98	0.11	0.27	4.15	0.15	3.56	0.33	5.86	4.26	7.96	0.43
High dose															
28	Mean	3.40	119.00	90.80	98.80	0.22	9.08	90.00	0.54	115.80	6.98	73.80	45.12	125.00	4.10
	SD	0.40	7.00	7.95	8.53	0.10	0.37	9.97	0.17	11.63	0.55	5.26	5.71	7.71	0.30
Female															
Control															
28	Mean	3.59	112.40	77.00	85.00	0.17	8.87	102.20	0.42	122.20	6.56	77.80	46.53	142.20	4.64
	SD	0.37	3.78	7.31	5.61	0.05	0.20	8.17	0.10	5.89	0.42	6.94	8.81	6.14	0.65
Low dose															
28	Mean	3.57	117.00	82.00	91.80	0.24	8.53	83.0*	0.20	117.00	7.06	80.20	41.88	116.40	3.96
	SD	0.35	6.75	5.83	4.32	0.05	0.29	7.04	0.06	6.04	0.25	2.59	1.59	4.39	0.53
Medium dose															
28	Mean	3.72	119.40	86.60	92.40	0.22	8.86	78.6**	0.22	112.80	6.66	77.40	40.88	122.00	3.96
	SD	0.46	6.02	11.46	10.45	0.04	0.35	7.64	0.08	7.92	0.28	4.04	0.76	9.54	0.21
High dose															
28	Mean	3.35	115.80	80.20	87.80	0.21	8.78	77.2***	0.24	114.20	6.78	79.40	41.75	123.00	3.92
	SD	0.21	6.26	7.82	7.40	0.08	0.42	5.54	0.06	5.89	0.45	7.02	1.14	2.45	0.15

Sprague-Dawley rats were individually treated with an oral dose of Aflapin at 0 mg/kg (control), 50 mg/kg, 250 mg/kg, or 2500 mg/kg daily for 28 days. Animals were sacrificed after 28 days of treatment. The values represent mean and SD, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, $n = 5$.

consecutive days in male and female SD rats. Aflapin was administered orally at doses of 0.0, 50, 250, and 2500 mg/ kg body weight per day.

Administration of Aflapin did not induce any statistically significant toxic effects. Based on the data from this study, the No Observed Adverse Effect Level (NOAEL) for Aflapin in male and female Sprague-Dawley rats is more than 2500 mg/ kg, and thus Aflapin may be considered as safe and non-toxic.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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