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Research Article

IN VIVO DERMAL ABSORPTION AND SUB-ACUTE TOXICITY STUDIES OF ESSENTIAL OIL FROM BLUMEA ERIANTHA DC

PRAJAKTA P. PEDNEKAR[a], ROHIT V. DHUMAL[b], AJIT G. DATAR[a], GEETA R. VANAGE[b]

[a]Guru Nanak Institute for Research and Development, G. N. Khalsa College, Matunga, Mumbai 400019, [b]National Center for Preclinical Reproductive and Genetic Toxicology, National Institute for Research in Reproductive Health, Parel, Mumbai 400012, India. Email: ajitgdatar@rediffmail.com

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ABSTRACT

Objective: The present investigation describes *in vivo* dermal absorption and sub acute dermal toxicity (28 days) study of essential oil from *Blumea eriantha* DC (*Compositae*).

Methods: The essential oil was extracted by hydrodistillation using Clevenger type apparatus. The dermal absorption studies were conducted in rabbits at dose of 15% (v/v) while sub acute toxicity was carried out in adult Holtzman rats at doses of 3%, 9% and 15% (v/v). The *in vivo* dermal absorption of essential oil was examined by means of Head space GC MS.

Results: The *in vivo* dermal absorption did not show presence of any essential oil component in the rabbit plasma samples. Further the sub acute treatment with essential oil did not show significant differences in the body weight, food consumption, hematological and biochemical parameters examined in both female and male groups. Necropsy and histopathological examination did not reveal any treatment related changes in treated groups. However, during the first week of study skin irritation was observed only in animals treated with high dose (15%) of essential oil which receded with time.

Conclusion: These findings suggest that the sub-acute dermal application of the *Blumea eriantha* DC essential oil in rats did not reveal any significant toxic effects at 9%. However skin irritation observed in high dose (15%) group indicating possible toxicity of essential oil. Therefore caution should be exercised in the application of essential oil especially at high dose (15%) and above.

Keywords: *Blumea eriantha* DC, Essential oil, Dermal absorption, Head space GC MS, Sub acute dermal toxicity

INTRODUCTION

Establishing the pharmacological basis of the efficacy of essential oils is a constant challenge as the fragrances of these oils are complicated and difficult to characterize [1, 2, and 3]. The influence of essential oils on skin permeation is of particular interest. The therapeutic efficacy and safety of many drugs commonly used in clinical practice can be augmented by individualization of their dosage [4]. The same principle that governs the effect of prescription drugs also influences the action of essential oils. Hence it is important to establish markers which can be used to monitor the absorption and elimination process of the essential oils. The transdermal permeation of essential oil molecules is complex, involving many possible steps from initial application to their arrival in the systemic circulation. They have been shown to penetrate into and through the skin, where they exert local therapeutic effects [5, 6]. Recently considerable research is in progress on the use of essential oils and their constituents as transdermal permeation enhancers to improve drug permeation. Essential oils have been investigated as potential skin penetration enhancers e.g. Basil essential oil showed an enhancing activity for accelerating transdermal delivery of indomethacin [7]. Most of these investigations on transdermal permeation have focused on the terpenes from essential oils as permeation enhancers e.g. menthol, limonene and cineole were employed as natural enhancers to improve the skin penetration of drugs [8, 9].

Many aromatherapists and members of the public consider natural essential oils to be completely safe. This is based on the misconception that all herbs are safe – because they are 'natural'. The dramatic increase in concentration of the essential oil compared with that in the whole plant (often the yield is 0.01%) demonstrates that essential oils are highly concentrated and are not equivalent to the whole herb [10]. Along with that they are lipophilic in nature and hence can pass across the membranes very efficiently [10]. As these properties are beneficial for their medicinal effects, this may also lead to their toxicity. The reported essential oil hazard includes allergic contact dermatitis, photosensitization, neurotoxicity, carcinogenicity [11]. Hence it is very important to study dermal absorption and toxicity of essential oils *in vivo*.

Blumea eriantha DC (*Compositae*) (*B. eriantha*) is an Indian medicinal plant. It is an erect, puberulous, aromatic, perennial herb commonly found abundantly along road sides, degraded forest lands. It is distributed mainly in Bihar, Karnataka, Maharashtra, Madhya Pradesh, Uttar Pradesh and Orissa in India [12]. The essential oil obtained from *B. eriantha* possesses significant antibacterial and antifungal properties [13] and may be incorporated in dermatological medicaments [14]. The antibacterial potential of essential oil from *B. eriantha* was confirmed by carrying out *in vitro* antibacterial assay [15]. The oil was subjected to *in vitro* antimicrobial testing against skin pathogens mainly acne inducing bacteria by means of broth microdilution method and time kill analysis. The essential oil displayed strong antibacterial activity against *Propionibacterium acnes* (MIC 0.39%), *Staphylococcus epidermidis* (MIC 1.56%), *Staphylococcus aureus* (MIC 0.19%) and *Streptococcus pyogenes* (MIC 0.09%). Further the essential oil was also studied for its chemical composition by Gas Chromatography Mass Spectrometry and antioxidant potential [16]. Total 72 components were identified in the essential oil out of which Ocim-(4E, 6Z)-ene <allo->, Caryophyllene <(E)->, Caryophyllene oxide, Carvotanacetone, Pinene <alpha->, Eudesmol <7-epi-alpha-> are major constituents. The oil showed activity as a radical scavenger at 437.92 ± 4.22µg/ml.

The obtained data for antimicrobial efficacy highlight the potential of *B. eriantha* essential oil as a natural antimicrobial agent in the treatment of skin infections and it is evident that it needs to be applied topically in a repeated dose. Hence sub acute dermal toxicity (28 days) studies were conducted to find out toxicity of essential oil on repeated dose. Further the GC-MS analysis showed that the essential oil from *B. eriantha* contains approximately 67% of the terpene hydrocarbons. This is indicative of its skin absorption potential as many of the terpenes are already in use as transdermal penetration enhancers. Hence dermal absorption elimination study was carried out with the aim to develop an initial database on the absorption of the essential oil *in-vivo*. Till date the dermal absorption elimination and toxicity studies on essential oil from *B. eriantha* are not reported. The data obtained from these studies will

be helpful in further clinical application of essential oil as topical antimicrobial agent.

MATERIALS AND METHODS

Plant Material

The entire upper portion including aerial part, stem and leaves of *Blumea eriantha* DC were collected from Seawoods, Navi Mumbai, Maharashtra, India between the months of December to March. The identification of the plant was done at the Blatter Herbarium, St. Xavier's College, Mumbai, India, by Dr. Rajendra Shinde. The plant specimen matches with the Blatter Herbarium specimen number PD 496 of P. Divakar.

Extraction of essential oil

The fresh plant material including aerial part, stem and leaves of *B. eriantha* were chopped into small pieces. 750g of plant material was subjected to hydrodistillation using Clevenger type apparatus of capacity 5 Liters. 3 liters of water was added to the plant material. The mixture was heated on heating mantle at 85^{0}C. The distillation was continued for three hours. The essential oil obtained was dried over anhydrous Sodium sulphate and stored at 4^{0}C in sealed vials until analysis. The yield of essential oil ranged from 0.14% to 0.25% (v/w) on a fresh weight basis.

Animals

The experimental protocol involving animals was carefully reviewed and approved by Institutional Animal Ethics Committee of National Institute for Research in Reproductive Health (NIRRH), where the studies were executed in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), India with approval number NIRRH/IAEC/36-11. Male and female (one each) New Zealand Strain Albino Rabbits were used for *in vivo* dermal absorption study. The body weight for male was 3.3 kg and that of female was 3 Kg. For sub-acute toxicity studies 8-10 week old, healthy adult Holtzman rats with the average body weight of 420gm for males and that of female around 250gm respectively were used. The rats were bred at NIRRH and were housed in polypropylene cage containing autoclaved corn cobb as bedding that was replaced on a weekly basis. Throughout the study, rats were provided with soy-free, in-house-prepared rat pellets (consisting of crude protein, fiber and nitrogen free extract) prepared at the institute and filtered drinking water (purified by UV and reverse osmosis), *ad libitum*. All the animals were maintained at the controlled temperature of 23±1^{0}C, humidity of 55± 5%, in a 14 h light/10 h dark cycle.

Dosage

For the *in vivo* dermal absorption study 15% of the essential oil of *B. eriantha* prepared in DMSO (Molecular Biology Grade, Himedia, India) was used. While for the sub-acute- toxicity study various concentrations such as 3%, 9% and 15% of the essential oil from *B. eriantha* prepared in DMSO (Molecular Biology Grade, Himedia, India) were used. These concentrations were selected based on the MIC value of antimicrobial study.

In Vivo Dermal Absorption Study

Approximately 24 hours before the test, an area (approximately 4 X 3 cm^2 area) on the dorsal surface of the rabbit skin was clipped free of hair for the application of test substance (see **Figure 1**)**.** 0.2 ml of 15% of the essential oil from *B. eriantha* was applied to this area. Approximately 1ml of blood sample was withdrawn from the central ear artery at predetermined times over 24 hr at 2, 6, 12, 24 hr. Blood was centrifuged to obtain plasma and was stored at −20^{0}C prior to analysis. The samples were then analyzed using headspace GCMS.

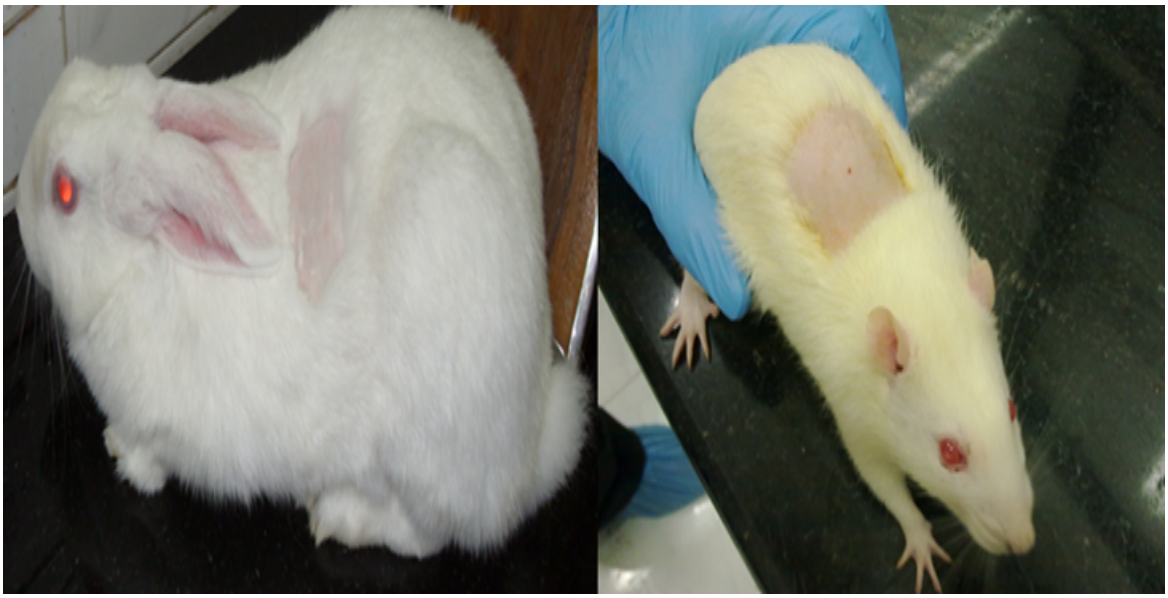

Fig. 1: Patch on Dorsal Surface of rats and rabbit.

Head Space Sampling and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The plasma samples were analyzed by GC-MS using Shimadzu GCMS QP-2010 Ultra system and Teledyne Tekmar HT3 Headspace analyzer. The system was equipped with fused silica Rtx-5 Sil MS silarylene capillary column with dimensions 30m X 0.25mm X 0.25µm. Helium (1ml/min) was used as a carrier gas. The conditions used for Headspace are given in **Table 1.** The program used for GC oven temperature was 1 minute isothermal at 50^{0}C, followed by 50-220^{0}C at a rate of 5^{0}C/min, then held at 220^{0}C for 1minute. Injector temperature was 266^{0}C. Helium was used as a carrier gas, at a flow rate 0.93 cm^3/ min. Split ratio was 1:40. The ionization of sample components was performed in the E.I. mode (70eV). The conditions for MS are given in **Table 1.** The Linear Retention Indices (LRI) for the obtained compounds were determined by co-injection of the sample with a solution containing the homologous series of C_8-C_{29} *n*-alkanes. Individual constituents were identified by referring to compounds known in the literature data and also by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 11), Flavor and Fragrance Natural and Synthetic Compounds mass spectral library database (FFNSC-2).

Table 1: Headspace and Mass Spectrometry conditions.

Head Space Conditions		MS Conditions	
Valve Oven Temperature	95^{0}C	Ion Source Temp.	200.00^{0}C
Transfer Line Temperature	110^{0}C	Interface Temp.	260.00^{0}C
Platen/Sample Temperature	80^{0}C	Solvent Cut Time	1.00 min
Sample Equilibration Time.	25.00 min	Detector Gain	0.84
Pressurize	8 PSIG	Start m/z	40.00
Pressurize Time	2.00 min	End m/z	500.00
Pressurize Equilibration Time	0.20 min	ACQ Mode	Scan
Loop Fill Pressure	6 PSIG		
Loop Fill Time	2.00 min		
Injection Time	1.00 min		

Sub-acute Toxicity Study (OECD guideline 410, 1995) [17]

The method was performed according to the OECD test guidelines 410 (OECD, 1995) [17]. The animals, five males and five females (*n* = 10/group), were assigned to the following four test groups namely Group I (vehicle control; DMSO), Group II (Low Dose: 3% of essential oil), Group III (Mid Dose: 9% of essential oil) and Group IV (High Dose: 15% of essential oil). A satellite group or recovery Group V (Recovery High Dose: 15% of essential oil) containing 5 males and 5 females were also included which was sacrificed 15 days after the termination of study. Approximately 24 hours before the test, an area (approximately 4 X 3 cm^2 area) on the dorsal surface of the rat skin was clipped free of hair for the application of test substance **(Figure 1)**. Animals were treated with 0.2 ml of vehicle (DMSO) or *B. eriantha* essential oil at doses of 3%, 9% and 15%, once daily for 28 consecutive days. Throughout the dermal application, the animals were examined for any clinical signs of morbidity, mortality, changes in body weight and food consumption.

Hematological and Biochemical Analysis.

At the end of the treatment, the animals were bled from the orbital sinus for clinical pathology assessment which included analysis of various hematology parameters. Hematological analysis was performed using an automatic hematological analyzer Abacus (Diatron).The parameters included: Red Blood Cell (RBC) Count, White Blood Cell (WBC) Count, Hemoglobin (Hb), Hematocrit (HCt), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Platelets count were determined. The differential leukocyte counting was performed with an optical microscopy after staining with Leishman's stain (Sigma-Aldrich) and, in each case, 100 cells were counted. For biochemical analysis, blood was centrifuged to obtain serum, which was stored at −20^{0}C until determination of the following parameters viz. Total Protein, Albumin, Globulin, Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), Cholesterol, Alkaline Phosphatase, Glucose, Creatinine, Urea, Uric Acid, Triglycerides, Bilirubin (Total And Direct), Calcium and Phosphorous. The serum biochemistry was performed by using fully automated serum biochemistry analyzer- EM 200 (ERBA) with biochemical kits from Spinreact, S. A. Ctra Santa Coloma, Spain.

Pathological Examination.

All the animals were sacrificed by CO_2 asphyxiation and necropsied for the gross evaluation of the various organs. The necropsy included careful and consistent dissection of various target organs like heart, liver, spleen, kidneys, lungs, brain, adrenal glands, testes, ovaries and application site (skin). These organs were carefully removed and weighed individually. Organ weights were expressed in terms absolute and relative (g/100 g of body weight). Finally the dissected tissues were fixed in 10% neutral buffered formalin, processed (Tissue processor Leica ASP300) and embedded (Paraffin Embedder Leica EG1150 H) in paraffin wax. Sections (5 µ) (Fully Automated Rotary Microtome Leica RM2255) of these tissues taken on glass-slides were stained using a combination of hematoxylin-eosin before observing under a microscope for histopathological evaluations.

Statistical analysis

For all the toxicological evaluations, the results of the treatment groups were compared with those of the control group. Data are expressed as the mean ± standard deviation (SD) for each group of animals at the number (*n*) indicated in tables. Statistical analysis was performed with two-tailed Student's t-test. Differences were considered significant at $P < 0.05$.

RESULTS

In the *in vivo* dermal absorption study, no essential oil constituent was detected in both male and female rabbit plasma samples at any of the time points till 24 hr. All animals from control and all the treated dose groups survived throughout the study period of 28 days. The animals did not exhibit any treatment related abnormal behavioral traits. Results of body weight determination of animals are tabulated in **Table 2** while results of food consumption are depicted in **Figure 2 and 3.**

No significant differences were found between the initial and final body weight of the rats treated with essential oil and vehicle. A similar absence of toxic effect was observed in the case of food consumption. These observations indicate the general and systemic well-being of the animals during the study period.

During the serum biochemistry investigations, there were no statistically significant differences between control and treated groups for the biochemical parameters measured except for increase in the levels of SGPT(P<0.05), cholesterol (P<0.05), total protein (P<0.05) in case of male rats while cholesterol (P<0.01) and total bilirubin (P<0.05) in case of female rats. These results are summarized in **Table 3 and 4.**

The hematological profile of the treated and control groups are tabulated in **Table 5 and 6.** The various hematological parameters of the treatment groups did not vary significantly from that of the control group, in animals of either sex. The results indicate the dermal safety of the essential oil to the various tissue systems.

The results of absolute and percent relative tissue weights are stated in **Tables 7 and 8.** The absolute organ weights other than liver, kidneys and brain were not altered by *B. eriantha* essential oil treatment compared to control. In case of percent relative organ weights alterations were found in liver, kidneys, spleen, epididymis and brain in case of males while in case of females the alterations were found in brain and heart. No treatment-related macroscopic findings were observed in treated animals at necropsy. For the histological investigation, no pathological changes were observed in the skin of animals in any group **(Figure 4)**. Similarly the histopathology of various target organs like heart, liver, spleen, kidneys, lung, ovaries and testis (**Figure 5)**, post necropsy, revealed that the natural architecture of the various organs remained unaffected. No dose related toxicity lesions were observed in organs including liver spleen, brain, lung, heart, kidneys, ovaries, testis as compared with the corresponding organs of the controls.

Table 2: Weekly body weight of animals in various treatment groups.

Male Average Body Weight in gm					
Weeks	**Control**	**Low**	**Mid**	**High**	**Recovery**
Initial	406.4 ± 2.70	391.4 ± 10.64	465.0 ± 18.80	418.4 ± 4.04	423.6 ± 13.30
1st Week	409.0 ± 11.31	393.4 ± 4.56	445.8 ± 20.62	412.6 ± 3.05	421.6 ± 21.17
2nd Week	414.2 ± 12.62	396.4 ± 5.03	446.0 ± 25.49	415.4 ± 3.85	426.4 ± 15.03
3rd Week	425.0 ± 16.61	396.6 ± 29.45	456.0 ± 23.70	426.2 ± 5.40	434.2 ± 13.48
4th Week	428.8 ± 18.74	390.4 ± 45.13	458.4 ± 20.56	424.8 ±7.43	447.6 ± 9.84
Female Average Body Weight in gm					
Weeks	**Control**	**Low**	**Mid**	**High**	**Recovery**
Initial	237.4 ± 3.13	259.4 ± 12.84	281.8 ± 10.55	257.2 ± 0.84	246.8 ± 7.26
1st Week	233.6 ± 1.14	260.4 ± 4.93	273.8 ± 11.32	252.6 ± 9.32	247 ± 12.02
2nd Week	237.8 ± 2.17	266.4 ± 7.09	275.2 ± 11.34	244.6 ± 6.47	249.8 ± 10.62
3rd Week	244.0 ± 4.30	264.8 ± 7.43	282.2 ± 8.84	257.8 ± 5.36	259.6 ± 12.18
4th Week	246.0 ± 2.00	267.2 ± 8.90	282.8 ± 11.54	259.2 ± 3.03	263.6 ± 18.08

Data are expressed as mean ± SD (n=5)

Where *p=<0.05, **p<0.01, ***p<0.001 against control group

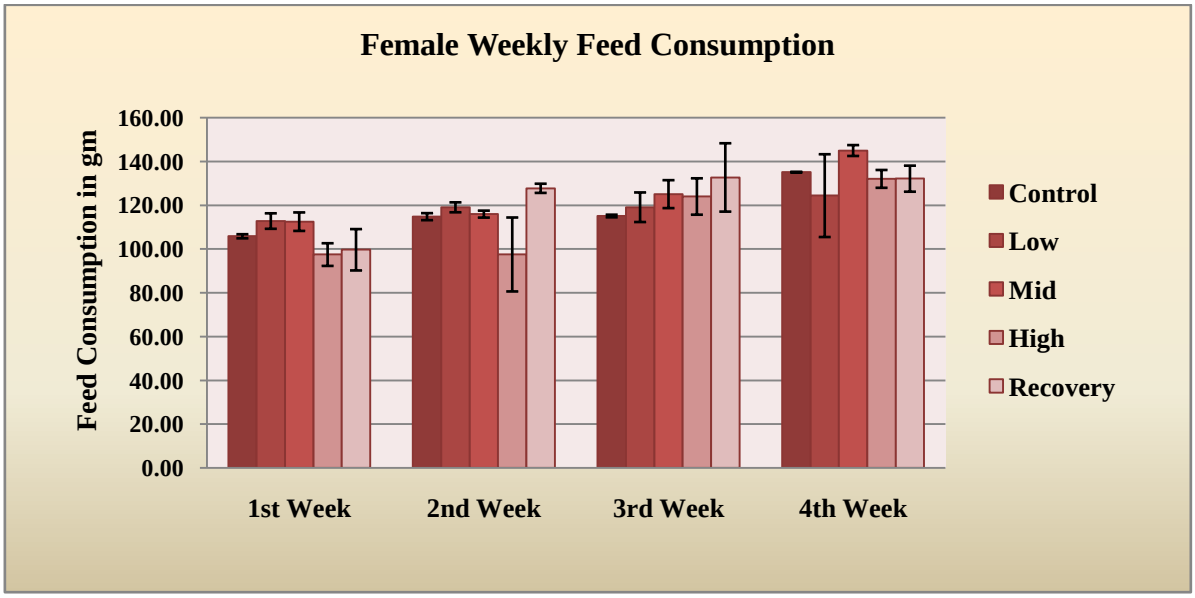

Fig. 2: Weekly Feed Consumption of Female Rats

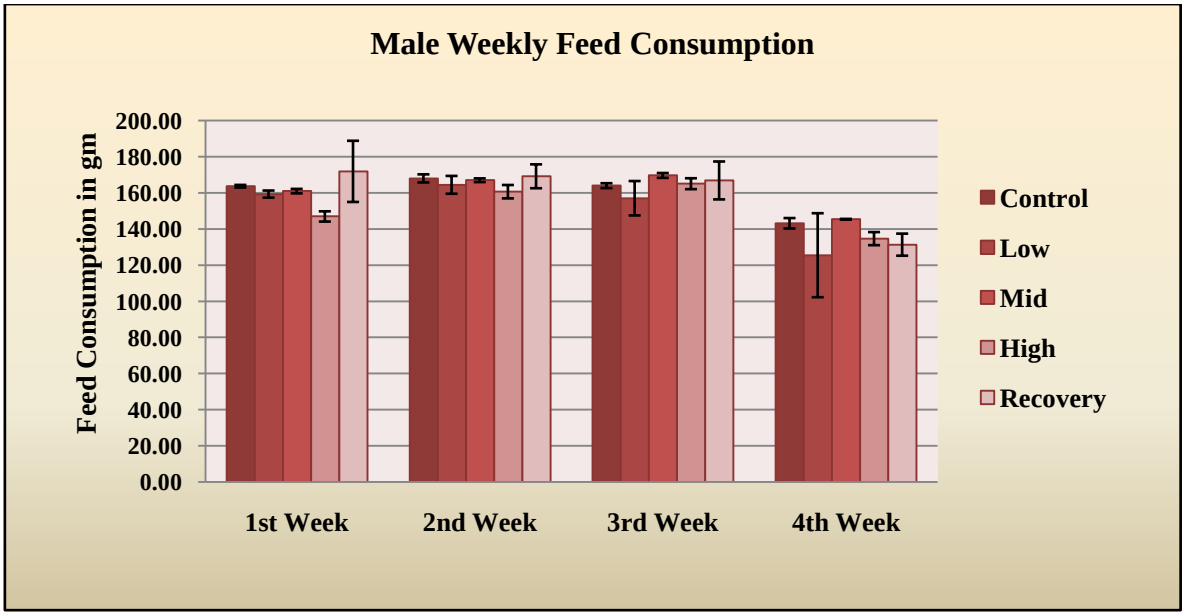

Fig. 3: Weekly Feed Consumption of Male Rats

Table 3: Biochemistry parameters analyzed for male rats in various treatment groups.

Parameters Analyzed	Control	Low	Mid	High	Recovery
SGPT (IU/L)	64.10±9.49	64.44±6.34	78.36±7.97*	66.62±14.37	67.12±30.66
SGOT(IU/L)	121.48±21.89	133.98±15.57	119.98±8.78	109.94±16.75	109.84±15.43
Phosphorus (mg/dl)	5.46±1.56	5.31±0.96	5.52±0.64	4.93± 1.18	5.41±1.77
Direct Bilirubin (mg/dl)	0.06±0.01	0.07±0.01	0.04±0.03	0.05±0.02	0.07±0.01
Total Bilirubin (mg/dl)	0.09±0.02	0.09±0.01	0.10±0.01	0.08±0.02	0.10±0.02
Urea (mg/dl)	36.38±3.17	35.26±4.24	37.82±2.87	33.98±4.72	36.74±5.34
Creatanine (mg/dl)	0.95±0.06	0.92±0.04	0.93±0.07	0.88±0.02	0.92±0.14
Glucose (mg/dl)	75.74±12.37	80.00±4.27	60.96±11.03	68.92±14.07	76.12±9.92
Triglyceride (mg/dl)	89.60±16.79	73.00±8.75	102.00±25.03	103.00±31.84	73.40±24.89
Cholesterol (mg/dl)	45.20±9.09	52.40±8.23	68.40±13.15*	61.40± 8.88*	58.20±11.78
Uric Acid (mg/dl)	2.40±1.13	1.80±0.34	2.33±0.71	1.88±0.68	1.82±0.40
Calcium (mg/dl)	9.72±0.41	9.76 ±0.39	9.56±0.34	9.80±0.57	9.86±1.27
Albumin (g/dl)	4.16±0.54	4.76±0.32	4.76±1.07	11.47±5.87	14.77±0.71***
Total Protein (g/dl)	6.33±0.65	6.14±0.46	7.22±0.40*	6.97±0.96	6.30 ± 0.73

Data are expressed as mean ± SD (n=5)

Where *p=<0.05,**p<0.01,***p<0.001 against control group

Table 4: Biochemistry parameters analyzed for female rats in various treatment groups.

Parameters Analyzed	Control	Low	Mid	High	Recovery
SGPT (IU/L)	51.88±9.87	59.72±4.96	56.56±7.77	53.72±18.59	51.54 ±8.69
SGOT(IU/L)	136.88±11.15	131.30±10.76	145.84±12.82	163.04±28.87	152.14±17.93
Phosphorus (mg/dl)	6.69±0.75	7.29±0.54	7.54±0.47	6.28±0.73	6.95±0.93
Direct Bilirubin (mg/dl)	0.03±0.03	0.03±0.03	0.02±0.02	0.04±0.01	0.03±0.01
Total Bilirubin (mg/dl)	0.06±0.01	0.08±0.02	0.07±0.04	0.08±0.01*	0.07±0.01
Urea (mg/dl)	36.7±2.18	33.74±3.23	37.86±5.06	15.5±2.54	33.5±2.53
Creatanine (mg/dl)	0.86±0.03	0.86±0.06	0.87±0.04	0.87±0.05	0.81±0.05
Glucose (mg/dl)	80.06±21.08	84.5±15.89	89.86±8.59	78.48±9.52	71.55±13.44
Triglyceride (mg/dl)	111.20±24.85	140.60±16.35	102.60±31.67	103.00±25.54	105.80±27.18
Cholesterol (mg/dl)	92.40±7.33	97.40±13.35	74.40±7.23**	78.20±17.51	106.60±20.38
Uric Acid (mg/dl)	1.97±0.44	2.21±0.54	1.93±0.52	1.81±0.54	1.79±0.35
Calcium (mg/dl)	9.56±0.59	10.06±0.30	9.28±0.37	9.06±0.15	9.52±0.96
Albumin (g/dl)	4.09±0.26	4.54±0.39	3.98±0.44	4.42±0.46	4.03±0.38
Total Protein (g/dl)	8.38±0.24	8.29±0.25	7.85±0.67	8.00±0.35	8.84±0.52

Data are expressed as mean ± SD (n=5)

Where *p=<0.05,**p<0.01,***p<0.001 against control group

Table 5: Hematology parameters analyzed for male rats in various treatment groups.

Parameters Analyzed	Control	Low	Mid	High	Recovery
Hemoglobin (g/dl)	16.46± 0.36	17.06± 0.61	16.60± 0.76	16.68± 0.54	16.70± 0.70
WBC (X 10^3/cmm)	11.77± 2.44	12.39± 4.83	15.46± 3.08	12.88±2.70	14.52± 3.63
RBC (X 10^6/cmm)	11.54± 0.46	11.58± 0.98	11.84± 0.68	11.84± 0.61	11.76± 0.67
PVC (%)	55.94±1.23	58.18±2.94	56.34±3.26	57.08±2.50	56.86±3.49
MCV (pg)	48.40±0.89	48.40±0.89	47.60±0.55	48.20±0.84	52.00±0.71
MCH (fl)	14.16±0.26	14.26±0.58	14.00±0.22	14.06±0.35	14.14±0.37
MCHC (g/dl)	29.38±0.20	29.32±0.76	29.46±0.38	29.22±0.38	29.4±0.66
Platelet (X 10^5/cmm)	578.60±67.47	451.60±191.18	538.20±194.74	603.00±237.39	414.40±245.50
Neutrophil (%)	36.80±7.40	35.00±4.18	38.00±6.28	33.20±7.82	32.00±6.60
Lymphocyte (%)	58.60±7.27	61.80±2.77	59.40±6.62	63.40±8.02	64.00±4.47
Monocyte (%)	2.80±1.64	1.80±1.30	1.60±1.14	2.00±1.58	2.40±1.52
Eosinophil (%)	1.80±1.48	1.40±1.14	1.00±0.71	1.40±1.14	1.60±1.52

Data are expressed as mean ± SD (n=5)

Where *p=<0.05,**p<0.01,***p<0.001 against control group

Table 6: Hematology parameters analyzed for female rats in various treatment groups.

Parameters Analyzed	Control	Low	Mid	High	Recovery
Hemoglobin (g/dl)	16.62±0.83	16.34±0.62	15.92±0.60	16±0.22	15.24±1.48
WBC (X 10^3/cmm)	8.21±1.75	9.39±2.95	8.43±1.61	8.81±0.82	7.6±3.38
RBC (X 10^6/cmm)	10.41±0.53	10.24± 0.34	9.97± 0.24	10.12± 0.20	9.49±1.14
PVC (%)	54.62± 2.91	53.24±1.74	51.92±1.65	52.96±0.83	50.44±5.37
MCV (pg)	52.40±0.55	51.80±0.45	52.00± 0.71	52.00± 0.71	53.40± 2.61
MCH (fl)	15.90±0.19	15.96±0.15	15.88±0.35	15.5±0.32	16.12±1.13
MCHC (g/dl)	30.44± 0.28	30.72±0.27	30.64±0.32	30.18±0.41	30.24±0.71
Platelet (X 10^5/cmm)	739.4± 233.84	501.8±183.28	577.6 ± 91.25	560.2 ±135.81	545.2±262.070
Neutrophil (%)	27.60±7.99	28.40±3.71	28.80± 6.76	30.80±6.30	36.80±11.19
Lymphocyte (%)	69.00±7.71	68.00±4.30	67.00±7.31	66.20± 6.94	59.60±12.01
Monocyte (%)	2.20±0.84	2.40±1.14	2.80±1.92	1.80±1.48	2.60±2.70
Eosinophil (%)	1.20±1.10	1.20±0.84	1.40±1.14	1.20±0.84	1.00±0.71

Data are expressed as mean ± SD (n=5)

Where *p=<0.05, **p<0.01, ***p<0.001 against control group

Table 7: Absolute Organ weight of animals in various treatment groups

Male Absolute Organ Weight in gm					
Organs	**Control**	**Low**	**Mid**	**High**	**Recovery**
Liver	16.96 ±1.78	15.72 ± 3.72	18.58 ± 1.73	19.45 ± 0.87*	19.94 ± 0.94*
Kidneys	3.62 ± 0.24	3.57 ± 0.40	4.14 ± 0.23*	3.89 ± 0.40	3.80 ± 0.33
Heart	1.65 ± 0.10	1.55 ± 0.10	1.70 ± 0.09	1.89 ± 0.32	1.75 ± 0.05
Adrenal Glands	0.13 ± 0.04	0.32 ± 0.45	0.30 ± 0.34	0.10 ± 0.03	0.10 ± 0.04
Testes	4.05 ± 0.21	3.89 ± 0.39	4.11 ± 0.36	3.93 ± 0.48	4.14 ± 0.29
Epididymis	1.56 ± 0.15	1.73 ± 0.21	1.88 ± 0.28	1.76 ± 0.16	1.85 ± 0.29
Spleen	1.06 ± 0.17	1.02 ± 0.08	1.18 ± 0.11	1.09 ±0.11	1.05 ±0.08
Brain	2.08 ± 0.13	2.11 ± 0.29	2.33 ± 0.11*	2.24 ± 0.17	2.34 ± 0.24
Female Absolute Organ Weight in gm					
Liver	9.92 ± 0.57	11.03 ± 0.71*	12.07 ± 0.84**	10.36 ± 0.91	10.63 ± 1.08
Kidneys	2.28 ± 0.11	2.44 ± 0.18	2.61 ± 0.12**	2.42 ± 0.13	2.49 ± 0.27
Heart	1.11 ± 0.06	1.07 ± 0.10	1.19 ± 0.08	1.04 ± 0.14	1.16 ± 0.08
Adrenal Glands	0.67 ± 0.38	0.37 ± 0.48	0.4 ± 0.42	0.53 ± 0.35	0.09 ± 0.09
Ovaries	0.21 ± 0.02	0.21 ± 0.02	0.51 ± 0.26	0.25 ± 0.04	0.31 ± 0.13
Uterus	0.92 ± 0.28	0.82 ± 0.24	1.09 ± 0.29	1.09 ± 0.33	1.30 ± 0.44
Spleen	0.77 ± 0.07	0.85 ± 0.04	0.78 ± 0.03	0.75 ± 0.07	0.81± 0.13
Brain	1.98 ± 0.07	1.98 ± 0.15	2.09 ± 0.07	1.97 ± 0.05	1.87 ±0.44

Data are expressed as mean ± SD (n=5)

Where *p=<0.05, **p<0.01, ***p<0.001 against control group

Table 8: Relative Organ weight of animals in various treatment groups.

Male Relative Organ Weight in %					
Organs	**Control**	**Low**	**Mid**	**High**	**Recovery**
Liver	3.95 ± 0.34	3.01 ±1.69	4.05 ± 0.21	4.58 ± 0.16*	4.45 ± 0.15*
Kidneys	0.84 ± 0.05	0.92 ± 0.03*	0.90 ± 0.06	0.91± 0.08	0.85 ± 0.06
Heart	0.39 ± 0.03	0.40 ± 0.03	0.37 ± 0.01	0.44 ± 0.07	0.39 ± 0.01
Adrenal Glands	0.0 3± 0.01	0.0 7 ± 0.1	0.06 ± 0.07	0.20 ± 0.14	0.02 ± 0.01
Testes	0.95 ± 0.05	1.00 ± 0.10	0.90 ± 0.08	0.92 ± 0.10	0.92 ± 0.05
Epididymis	0.36 ± 0.02	0.44 ± 0.01**	0.41 ± 0.08	0.41 ± 0.03*	0.50 ± 0.18
Spleen	0.25 ± 0.04	0.27 ± 0.04*	0.26 ± 0.03	0.26 ± 0.03	0.24 ± 0.01
Brain	0.49 ± 0.05	0.55 ± 0.12	0.51± 0.04*	0.52 ± 0.04	0.52 ± 0.06
Female Relative Organ Weight in %					
Liver	4.03 ± 0.24	4.19 ± 0.30	4.29 ± 0.35	4.00 ± 0.33	3.93 ± 0.31
Kidneys	0.93 ± 0.05	0.91± 0.04	0.93 ± 0.04	0.94 ± 0.05	0.93 ± 0.09
Heart	0.45 ± 0.03	0.40 ± 0.03*	0.42 ± 0.03	0.40 ± 0.05	0.44 ± 0.04
Adrenal Glands	0.22 ± 0.18	0.09 ± 0.07	0.14 ± 0.15	0.02 ± 0.01	0.03 ± 0.01
Ovaries	0.09 ± 0.01	0.08 ± 0.01	0.18 ± 0.08	0.10 ± 0.02	0.11 ± 0.03
Uterus	0.37 ± 0.11	0.31 ± 0.09	0.39 ± 0.11	0.42 ± 0.13	0.41 ± 0.058
Spleen	0.31± 0.03	0.31 ± 0.09	0.28 ± 0.02	0.29 ± 0.02	0.31± 0.03
Brain	0.81± 0.03	0.74 ± 0.04*	0.74 ± 0.03*	0.76 ± 0.02*	0.71± 0.16

Data are expressed as mean ± SD (n=5)

Where *p=<0.05, **p<0.01, ***p<0.001 against control group

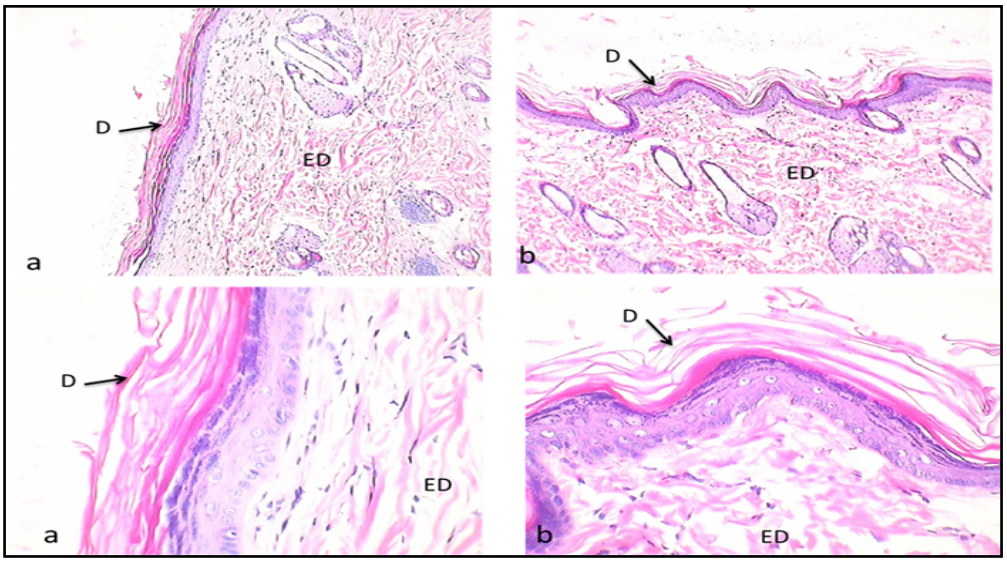

Fig. 4: Histomorphology of skin: (a) skin (Vehicle control), (b) skin (15%). The skin sections were taken at 4-5 µm and stained with haematoxylin and eosin (10X). Abbreviations: D: Dermis; ED: Epidermis

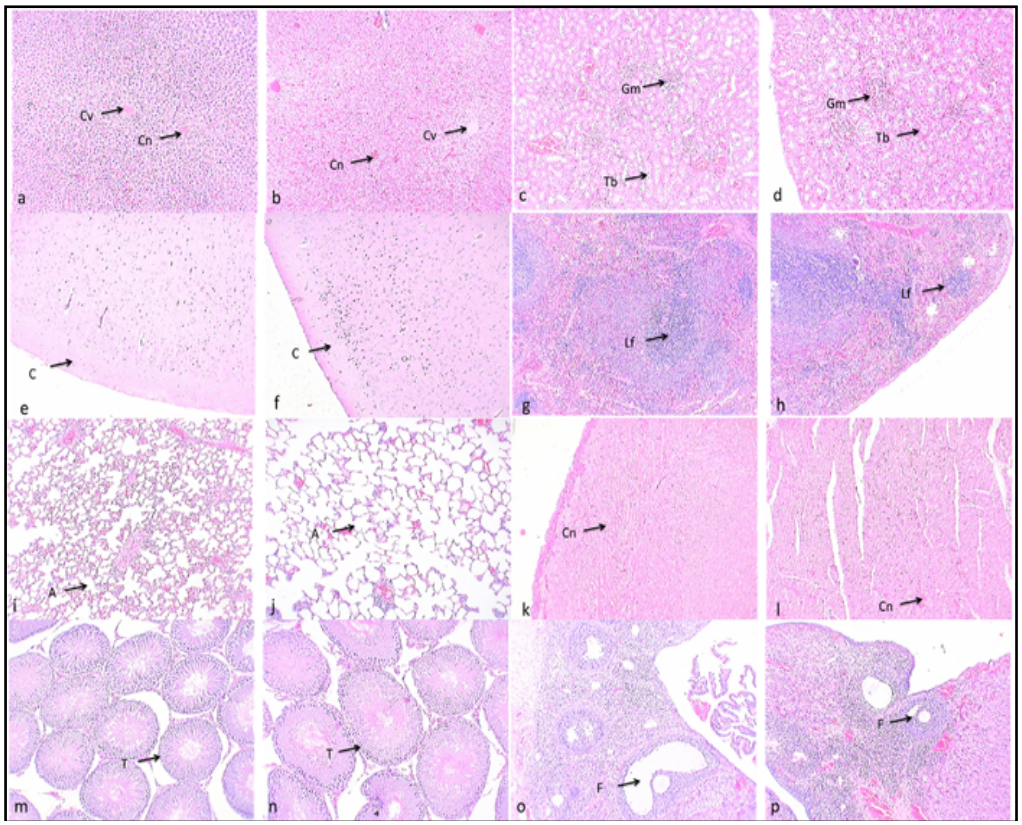

Fig. 5: Histomorphology of: (a) liver (Vehicle control), (b) liver (15%), (c) kidney (Vehicle control), (d) kidney (15%), (e) Brain (Vehicle control), (f) Brain (15%), (g) spleen (Vehicle control), (h) spleen (15%), (i) Lung (Vehicle control), (j) lung (15%) , (k) heart (Vehicle control), (l) heart (15%), (m) Testis (Vehicle control), (n) Testis (15%) (o) Lung (Vehicle control), (p) lung (15%) The various organ sections were taken at 4-5 µm and stained with haematoxylin and eosin (10X). Abbreviations: Cn: Congestion; Cv: Central Vein; Lf: Lymphoid follicle; Gm: Glomerulus; Tb: Tubule; A: Alveoli; c: cortex; A: alveoli; T: Tubule; F: follicle.

DISCUSSION

A biological sample containing phytoconstituents require sample pretreatment in order to isolate phytoconstituents from interfering matrix substances, to liberate the phytoconstituents from the protein binding sites, to concentrate the phytoconstituents for more sensitive analysis and to separate the phytoconstituents from metabolites. Headspace analysis avoids sample pretreatment as well as loss of volatiles during sample pretreatment and also concentrates the analytes. Hence head space technique found to be fast and better technique, than any other extraction methods, for analysis of volatiles from plasma samples. In this study the specificity of mass spectrometry has been used for identification of essential oil constituents while headspace was used for direct injection of volatile constituents directly from the plasma. The *in vivo* dermal absorption of essential oil was examined by means of Head space GC MS which did not show presence of any essential oil component in the rabbit plasma samples hence, a sub-acute dermal toxicity study was carried out to check the local toxicity of the essential oil from *B. eriantha*. The doses employed in the present study (3%, 9% and 15% concentration of essential oil) represent up to 15 times more than the MIC values obtained in the antimicrobial studies. In order to ensure health status of animals, hematological and clinical chemistry was determined for all the animals included in the study before starting of the treatment period and at the termination. After application of the test material, during first week skin irritation was observed only in animals in the group treated with 15% of essential oil however irritation receded with time by the end of second week. Along with that histopathology analysis of skin did not reveal any toxic lesions or alteration in the natural architecture of the skin.

The absence of any significant differences in the body weight provides support for the safety of essential oil of *B. eriantha*. Also there were no significant changes in animal behavior and food consumptions in essential oil treated group at any dosage. These observations indicate no effects of the essential oil on the general well-being of the animals.

Blood forms the main medium of transport for many drugs and xenobiotics in the body and for that matter components of the blood such as red blood cells, white blood cells, haemoglobin and platelets are at least initially exposed to significant concentrations of toxic compounds. Damage to and destruction of the blood cells are detrimental to normal functioning of the body. Hence blood parameters analysis is relevant to risk evaluation. Essential oil of *B. eriantha* did not have any significant effect on the haematological parameters measured, suggesting low toxicity.

The biochemical evaluation is important since there are several reports of liver and kidney toxicity related to the use of phytotherapeutic products. Kidney is a sensitive organ, whose function is known to be affected by a number of factors such as drugs including phytochemicals of plant origin that ultimately lead to renal failure [18]. In preclinical toxicity studies, renal changes are particularly liable to occur because of the high doses given and the fact that the kidneys eliminate many drugs and their metabolites [19, 20]. Absence of significant differences in serum levels of creatinine and urea in the *B. eriantha* essential oil treated groups compared to controls indicates that the essential oil did not have any deleterious effects on renal function. This conclusion correlates well with findings from histopathological examination of the kidney as it did not indicate any significant essential oil induced cellular lesions. Serum bilirubin is also considered as a true test of liver function, since it reflects the liver's ability to take up, process, and secrete bilirubin into the bile. There were no elevations in direct and total bilirubin fractions after treatment with the essential oil. However, histopathological assessment data revealed some mild congestion in liver ultrastructure at highest dose of essential oil (15%). The lack of marked changes in serum levels of direct and total bilirubin

fractions implies that the histopathological changes seen were not significant enough to affect liver function and may be considered as clinically unimportant. Although there was significant alterations in case of few organ weights in treated groups as compared to control, these organs histopathology did not reveled any abnormal changes so these changes can be considered as biological variations. Similarly in histopathology of various other target organs like spleen, brain, ovaries, testis no dose related toxicity lesions were observed in essential oil treated groups at any dosage.

Thus the sub-acute toxicity studies clearly indicate the dermal safety of the essential oil from *B. eriantha*. Since the dermal dose of 9% per day of *B. eriantha* essential oil administered for 28 consecutive days did not induce any biochemical, hematological, anatomical, and histopathological signs of toxicity, it can be taken as the no-observed adverse-effect level (NOAEL) for rats of both sexes under the experimental conditions used. This Information will help for future clinical studies of the medicinal safety and *in vivo* experimental studies of the pharmacological potentialities of this mode of administration of the essential oil. However, it should be emphasized that this NOAEL was derived from a subacute study only. Since toxicity in humans cannot always be entirely extrapolated from animal studies, clinical evaluation should be performed to precisely define the safe dosage.

CONCLUSION

In this study an analytical methodology was developed for the identification of constituents of essential oil from *Blumea eriantha* DC from rabbit blood plasma samples. The study demonstrates no systemic absorption of the essential oil constituents within 24 hours following its dermal application. The 28-day sub-acute toxicity studies demonstrated no treatment related adverse effects, no mortality and no significant weight loss was attributable to the application of *Blumea eriantha* DC essential oil in animals of either sex at the 3% and 9% concentration. The animals exhibited signs of good health and well-being throughout the study. Therefore, the NOAEL for the sub-acute toxicity study could be 9%. The results indicate the dermal safety of the essential oil of *Blumea eriantha* DC for prolonged application. Thus a safety and *in vitro* antimicrobial efficacy indicate its potential application as antimicrobial agent in the treatment of acne vulgaris and other skin infections.

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