Available online on 26.03.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

© 2011-18, publisher and licensee JDDT, This is an Open Access article which permits unrestricted non-commercial use, provided the original work is properly cited

Research Article

Evaluation of acute and subchronic toxicity of dragon blood resin extract

Saxena Gaurav*[1], Mittal Abhilasha[1], Siddiqui Abdul Wadood [2]

[1] NIMS, Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India

[2] Lloyd School of Pharmacy, LIMT, Gtr Noida, UP, India

ABSTRACT

Draceana cinnabari (Dracaenaceae) is traditionally used in the treatment of wounds, leucorrhea, fractures, and diarrhoea. Herein, we performed a pre-clinical safety evaluation of extract of *Draceana cinnabari* resin on Spradgue Dawley rats. Toxicological codicil of the extract was carried out following OECD guidelines 423 and 407, with minor changes. DC resin methanol extract administered to the rats by oral gavage at 50, 500, 1500, and 2500 mg/kg body weight daily up to 28 days to male and female rats. Herbal extract could most likely be very much endured up to the dose 2000 mg/kg body weight and could be named Category 4. Oral toxicity studies confirm that *Draceana cinnabari* has no therapy-related demise or toxic signs on rats. Therefore, the resin, ought to be suitably considered for additional research for its medicinal and therapeutic efficacy.

Keywords: Dracaenaceae, DC extract, Resin, Acute toxicity, Sub Chronic Toxicity

Article Info: Received 08 Feb 2019; Review Completed 09 March 2019; Accepted 19 March 2019; Available online 26 March 2019

Cite this article as:

Saxena G, Mittal A, Siddiqui, AW, Evaluation of acute and subchronic toxicity of dragon blood resin extract, Journal of Drug Delivery and Therapeutics. 2019; 9(2):362-366 **http://dx.doi.org/10.22270/jddt.v9i2.2435**

***Address for Correspondence:**

Gaurav Saxena, NIMS, Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India

INTRODUCTION

Medicinal plants, either as an extract, pure compound, or as a derivative, offer unlimited opportunities for the discovery of new drugs. Most of the natural products used in folk remedy have solid scientific evidence about their biological activities. However, there is little information or evidence available concerning the possible toxicity that medicinal plants may cause to consumers[1] . Toxicity is a terminology used to describe the property of being poisonous or indicate the state of detrimental outcomes by means of the interaction between toxicants and cells. This mechanism of action may likewise change depending on the cell membrane and chemical residences of the toxicants. It may occur inside the cell membrane or on the cell surface or tissue beneath as well as at the extracellular matrix. In most of the occurrence, visceral organs such as liver, lung and kidney are affected via the toxicants[2] .

Many studies have already shown that several medicinal plants have detrimental effects. The customary use of any plant for the ameliorative outcome, itself does not guarantee the safety of that plant. Thus, data of the acute and sub-chronic toxicity studies on medicinal plants or their preparations should be obtained to increase the confidence in their safety to humans, particularly for use in the development of pharmaceuticals[3].

Dragon's blood is a name applied to resins obtained from plants like Dracaena, Daemonorops, Croton and Pterocarpus. There are many types of chemical constituents in *Draceana cinnabari* (Dracaenaceae) resin (Figure 1), including flavones, saponins, steroids and volatile oils. [4,5]

Figure 1: *Dracaena cinnabari* resin

Pharmacological investigations indicate that it can inhibit the aggregation of platelets, venous thrombosis and bacterial action. It additionally has anti-inflammatory, analgesic and homeostatic effect.[6,7] Recently, *Dracaena* has been clinically used for treatment of cerebral arterial thrombosis, ischemic heart disease and peptic ulcer [8]. However, not much work has been done of its safety profile by toxicity studies The purpose of this work is to explore the acute and chronic toxicity profile along with the antioxidant properties of *D. cinnabari* resin extract.

MATERIALS AND METHODS

Plant material

Dracaena cinnabari resin used in this study was procured from the local bazaar Ballimaran, Delhi, India. A voucher specimen (NISCAIR/RHMD/Consult/2008-09/1192/224) of the resin was collected, identified and deposited at NISCAIR, New Delhi, India

Chemical reagents

All the chemicals used in this study were obtained from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), and SD Fine-Chem. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Preparation of plant extracts

The crude extracts were prepared by cold maceration technique [9]. The extraction was done by refluxing 300 g of resin in 2400ml of methanol (98%) and placing the mixture on an orbital shaker (at 160rpm) for 72 hrs. The mixture was concentrated in a rotary evaporator. The resulting extract (DRE) was lyophilised and stored in an amber coloured airtight container at 4°C until further use.

Selection and Procurement of animals

Experimental animals

For acute and subacute oral toxicology assessment, (total of 65), age (6 weeks) and weight (129.1 to 140.2 gm) matched female and male Sprague Dawley (SD) pathogen-free rats were used. Fifteen female rats were used for an acute oral toxicity test; furthermore forty rats (twenty-five males and twenty-five females) were used for acute oral toxicity tests (twenty-eight days of recurrent dose). The toxicity tests were carried out according to Organization for Economic Cooperation and Development (OECD) test guideline, specifically OECD Guideline 423 for the acute oral toxicity test[10] and OECD Guideline 407 for the sub-acute oral toxicity test[11] with slight modifications. All animals were procured and housed in animal house maintained under standard hygienic conditions. Animals were acclimatised to the laboratory environment for seven days ahead of the experiments. The animals were allowed free to access commercial rat pellet diet (Lipton India Ltd, Mumbai, India) and water *ad libitum*. The bedding materials of the enclosure were changed every day. All the experimental trial was carried out in conformity with the CPCSEA guidelines. The study design was approved by the Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) (Reg No. CPCSEA Reg. No. 1283/c/09/CPCSEA). Bhopal (MP), India. Protocol approval reference no. PBRI/11/IAEC/PN-209.

Acute toxicity

Animals were randomly divided in the group (n=3), a control group (Group A) and dose levels (Groups A_1, A_2, A_3 and A_4, administered respectively 50mg, 500mg, 1500mg and 2500 mg DRE / kg body weight). DRE was given once by oral gavage at a weight of 10 mL/kg body weight. The control animals have been treated with distilled water in the same volume. Animals were observed for three hours after dose administration changes in behaviour. The rats were weighed, and visual observations for mortality, behavioural pattern (salivation, fur, lethargy, and sleep), changes in physical appearance, injury, pain, and any signs of illness were conducted once daily during of 24 and 48 hours.

Sub-chronic Toxicity

The remaining forty male and female SD rats (n = 5 males and 5 females/group) were arbitrarily divided into a control (Group SC) and three dose teams (GroupSC_1, SC_2, SC_3, and SC_4 administered fifty, five hundred, fifteen hundred and twenty-five hundred mg/kg body weight/day, respectively). The standard procedures were followed as per the OECD 407 guidelines (OECD, 143 2008). DRE was administered once daily for four weeks, by oral gavage of 10 mL/kg body weight. Control animals have only been, treated with the same quantity of distilled water during the four weeks assay period.

The dosage was freshly prepared on a day to day basis. The rodents were weighed. Rats observed visually for morbidity, changes in behaviour (like dormancy, fur, rest and salivation), changes in physical appearance, damage, agony and malady were done once a day during that period. Animals were fasted overnight at the end of the treatment, albeit with water *ad libitum*. Blood samples were collected thru retro-orbital puncture employing capillary tubes for haematological and biochemical research along with anticoagulant EDTA. All rats were anaesthetised with isoflurane and afterwards sacrificed through exsanguinations from carotid and jugular vessels. [12] Complete postmortem examination was carried out on all animals.

Analogous organ weight determination:

Organs, that is liver and kidneys were meticulously eviscerated from the rats immediately after sacrifice and weighed in grams. The comparative organ weight of each animal was then quantified as follows:

Relative organ weight = absolute organ weight (g) × 100/ body weight of rat on sacrifice day (g)

Haematology and serum biochemistry:

Haematological and biochemical studies of liver function, plasma constituents, and electrolyte concentrations were determined using standard clinical procedures.

Haematological parameters

For complete blood count, 3.0 ml of venous blood was taken in a vacutainer tube containing EDTA as an anticoagulant. Basic haematological estimated were haemoglobin (Hb) content, red blood cell (RBC), total white blood count (WBC), and differential WBC count (neutrophil, eosinophil, basophil, lymphocyte and monocyte), as well as platelet count, were performed using an automated analyzer Automatic Hemato analyzer (MS-4) (Melet Schloesing Laboratories, Osny, France)).[13]

Serum biochemical parameters, which were measured from serum using kits (Span Diagnostic Ltd., Surat, Gujarat, India using a UV-VIS Spectrophotometer (Shimadzu, Tokyo, Japan). Serum biochemical parameters included total serum protein (TP), creatinine (CREA), blood urea nitrogen (BUN), albumin (ALB), alkaline phosphatase (ALP), glutamate oxaloacetate transaminase (GOT), and glutamate pyruvate transaminase(GPT)[14] .

Histopathology

Organs were fixed and preserved in 10% phosphate-buffered formalin. Tissues were fixed by embedding in paraffin, sectioned, and stained with hematoxylin and eosin. Collected tissues were grossly and microscopically examined[15].

Statistical analysis

The results were expressed as mean ± SEM (n = 5). Data acquired as of hematology analysis, serum biochemistry, as well as the body and organ weight measurements were articulated as mean + SD and evaluated by unpaired t test (2-tailed P value) to compare the mean of the treated group of each sex with the control.Data were analysed using Student's t-test, and results were considered significant when $p < 0.05$.

Table 1: General appearance and behavioural observations for control and treated groups in Acute toxicity study of DCE (n = 5).

Observation	**3 Hours**					24 hours					48 hours				
	A	A_1	A_2	A_3	A_4	A	A_1	A_2	A_3	A_4	A	A_1	A_2	A_3	A_4
Behavioural patterns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Coma	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Diarrhea	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Eyes	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Lethargy	*	*	*	*	*	*	*	*	*	*	*	*	*	*	--
Mucous	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Salivation	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Skin and fur	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Sleep	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Tremors	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Dosing Groups A=Control group A_1=5mg/kg group A_2=50mg/kg group A_3=300mg/kg group A_4=2000mg/kg group *= Normal --= not normal

Table 2: Effect of DRE on body weight (g), organ (liver and kidney) as well as relative organ weight of (female) rats (n = 5).

Group	Body Weight (g)				Absolute weight (g)		Relative weight (g/100g body weight)	
					Liver	Kidney	Liver	Kidney
	Day "0"	Day "07"	Day "14"	Day "28"	Day "28"	Day "28"	Day "28"	Day "28"
SC	125.4±4.5	183.5±7.3	231.9±8.4	289.6±5.7	6.37 ±0.51	0.89 ± 0.36	2.21 ±0.13	0.31±0.03
SC_1	126.1±5.1	182.7±4.8	230.7±8.6	288.8±7.7	6.35± 0.39	0.92 ± 0.42	2.20 ±0.11	0.32±0.02
SC_2	125.3±8.1	182.6±7.6	232.7±9.6	290.3±3.5	6.33± 0.10	0.89 ± 0.85	2.18 ±0.14	0.31±0.04
SC_3	123.2±6.1	184.2±1.8	230.1±3.7	289.1±6.3	6.40± 0.30	0.98 ± 0.40	2.14 ±0.12	0.34±0.02
SC_4	124.1±2.1	183.4±3.9	229.7±8.6	288.1±3.2	6.43± 0.25	0.92 ± 0.32	2.19 ± 0.13	0.32±0.03

Values are presented as the mean±standard deviation

Dosing Groups SC= Control SC_1 SC_2 SC_3 SC_4

Table 3: Effect of DRE on body weight (g), organ (liver and kidney) as well as relative organ weight of (male) rats (n = 5).

Group	Body Weight				Absolute liver weight (g)		Relative kidney weight (g/100g body weight)	
					Liver	Kidney	Liver	Kidney
	Day "0"	Day "07"	Day "14"	Day "28"	Day "28"	Day "28"	Day "28"	Day "28"
SC	153.4±6.5	236.5±8.3	310.9±7.4	394.6±4.9	10.73±0.18	1.34± 0.32	2.72±0.13	0.34 ± 0.01
SC_1	153.4±5.7	235.9±4.1	311.0±5.6	395.2±3.2	10.90±0.17	1.42± 0.45	2.76±0.12	0.36 ± 0.02
SC_2	154.1±6.3	235.9±4.1	310.5±7.9	394.3±5.7	10.53±0.19	1.33± 0.60	2.67±0.10	0.34 ± 0.01
SC_3	154.6±1.9	235.9±4.1	310.1±4.9	392.1±2.7	10.59±0.18	1.29± 0.55	2.70±0.14	0.33 ± 0.03
SC_4	152.7±9.7	234.6±3.9	309.7±6.7	393.7±3.5	10.82±0.24	1.37± 0.20	2.75±0.11	0.35 ± 0.01

Values are presented as the mean±standard deviation

Dosing Groups SC= Control SC_1 SC_2 SC_3 SC_4

Table 4: Hematological s data of female SD rats treated orally with DRE for 4 weeks (n = 5)

Group	EO (%)	Hb(g/dL)	LY (%)	MO (%)	Ne(%)	Plt ($10^3/\mu L$)	RBC ($10^6/\mu L$)	WBC ($10^3/\mu L$)
SC	1.9 ± 0.8	13.4 ± 0.4	78.7 ± 5.7	3.1 ± 1.2	15.8 ± 5.0	1071 ± 125	7.47 ± 0.33	3.17 ± 0.63
SC_1	2.1 ± 0.4	14.3 ± 0.3**	78.9 ± 4.5	3.1 ± 1.0	15.4 ± 4.3	990 ± 114	7.93 ± 0.28**	3.81 ± 1.03
SC_2	2.0± 0.6	14.0 ± 0.6*	77.2± 3.7	2.9 ± 0.4	15.0 ± 3.8	1020 ± 116	7.74 ± 0.16*	3.88 ± 1.06
SC_3	1.9 ± 0.5	13.8 ± 0.5	78.4 ± 4.6	3.4 ± 0.5	14.9 ± 4.1	1038 ± 123	7.63 ± 0.24	3.99 ± 1.07
SC_4	1.8 ± 0.7	13.9 ± 0.4*	80.0 ± 5.6	4.0 ± 1.5	14.8 ± 5.5	1040 ± 128	7.66 ± 0.20	4.05 ± 0.83

Values are presented as the mean±standard deviation

Dosing Groups SC= Control SC_1 SC_2 SC_3 SC_4

EO, Eosinophil; HB, Hemoglobin concentration; LY, Lymphocyte; MO, Monocyte; NE, Neutrophil;PLT, Platelet count; RBC, Red blood cell count, WBC, White blood cell count .

Table 5: Serum biochemical data of female SD rats treated orally with DRE for 4 weeks (n = 5)

Group	ALB (g/dL)	ALP (U/L)	ALT (IU/L)	AST (IU/L)	BUN (mg/dL)	CREA mg/dL)	TP (g/dL)
SC	3.1 ± 0.2	139 ± 28	34 ± 11	86 ± 22	16.3 ± 1.9	0.6 ± 0.0	6.8 ± 0.2
SC_1	3.0 ± 0.2	147 ± 24	36 ± 12	96 ± 26	15.8± 1.6	0.6 ± 0.0	6.6 ± 0.3
SC_2	2.8± 0.1	150 ± 24	33 ± 14	91 ± 31	15.5 ± 2.0	0.6 ± 0.0	6.7 ± 0.4
SC_3	2.9 ± 0.1	156 ± 35	30 ± 9	87 ± 18	15.2 ± 1.4	0.6 ± 0.1	6.8 ± 0.4
SC_4	3.1 ± 0.2	156 ± 35	30 ± 9	87 ± 18	15.2 ± 1.4	0.6 ± 0.1	6.7 ± 0.2

Values are presented as the mean±standard deviation

Dosing Groups SC= Control SC_1 SC_2 SC_3 SC_4

ALB, albumin; ALP, alkaline phosphatase; ALT, ala- nine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CREA, creatinine; TP, total protein;

Table 6 Hematological s data of male SD rats treated orally with DRE for 4 weeks (n = 5)

Group	EO (%)	Hb(g/dL)	LY (%)	MO (%)	Ne(%)	Plt ($10^3/\mu L$)	RBC ($10^6/\mu L$)	WBC ($10^3/\mu L$)
SC	1.3 ± 0.6	14.6 ± 0.7	80.4 ± 5.7	2.2 ± 1.1	15.7 ± 4.6	1001 ± 62	8.44 ± 0.37	6.94 ± 1.30
SC_1	1.4 ± 0.4	14.3 ± 0.5	79.2 ± 4.8	2.4 ± 0.6	16.6 ± 4.4	1006 ± 73	8.47 ± 0.25	7.26 ± 1.88
SC_2	1.5 ± 0.5	14.3 ± 0.9	77.6 ± 6.2	2.6 ± 0.4	17.7 ±5.6	990 ± 59	8.35 ± 0.21	6.42 ± 1.56
SC_3	1.6 ± 0.7	14.4 ± 0.3	76.3 ± 7.2	2.7 ± 0.9	18.9 ± 6.3	998 ± 78	8.27 ± 0.24	5.83 ± 1.56
SC_4	1.5 ± 0.6	14.5 ± 0.2	79.3 ± 4.8	2.4 ± 0.7	16.4 ± 3.9	1045 ± 160	8.46 ± 0.23	5.81 ± 0.95

Values are presented as the mean±standard deviation

Dosing Groups SC= Control SC_1 SC_2 SC_3 SC_4

EO, Eosinophil; HB, Hemoglobin concentration; LY, Lymphocyte; MO, Monocyte; NE, Neutrophil;PLT, Platelet count; RBC, Red blood cell count, WBC, White blood cell count .

Table 7: Serum biochemical data of male SD rats treated orally with DRE for 4 weeks (n = 5)

Group	ALB (g/dL)	ALP (U/L)	ALT (IU/L)	AST (IU/L)	BUN (mg/dL)	CREA mg/dL)	TP (g/dL)
SC	2.5 ± 0.1	287 ± 45	32 ± 4	101 ± 26	14.9 ± 2.1	0.6 ± 0.1	6.1 ± 0.2
SC_1	2.4 ± 0.1	285± 41	33 ± 6	95 ± 23	14.8 ± 1.7	0.6 ± 0.1	6.0 ± 0.2
SC_2	2.3 ± 0.6	284 ± 31	32 ± 4	95 ± 46	14.3 ± 2.3	0.6 ± 0.1	6.0 ± 0.2
SC_3	2.4 ± 0.1	296 ± 76	31 ± 6	96 ± 19	13.8 ± 2.1	0.6 ± 0.1	6.0 ± 0.2
SC_4	2.5 ± 0.1	276 ± 46	32 ± 8	95 ± 22	13.3 ± 1.5	0.5 ± 0.1	6.0 ± 0.3

Values are presented as the mean±standard deviation

Dosing Groups SC= Control SC_1 SC_2 SC_3 SC_4

ALB, albumin; ALP, alkaline phosphatase; ALT, ala- nine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CREA, creatinine; TP, total protein;

RESULTS

Acute Toxicological Studies

In the present investigation, we assessed the likely toxicity and 50% lethal dose (LD50) of DRE following a regiment of solitary oral to female rodents. DRE was orally administered once at doses of 50mg, 500mg, 1500mg and 2500 mg DRE / kg body weight to SD rats. Mortality, clinical signs, and body weight changes were observed for 120 hours days after administration. No deaths in any animals were observed during the experimental period. No test substance-related effects were evident concerning clinical signs, body weight changes, and necropsy findings at all dosages as shown in Table 1. These results suggest that acute exposure to DRE does not cause toxic effects, and its LD50 value is considered to be greater than 2500 mg/kg in rats.

Sub-chronic Toxicological Studies

No toxicity signs (such as piloerection, alteration in the locomotor activity or food and water consumption) or deaths were recorded during the 28 consecutive days of treatment by oral route with *D. cinnabari* resin extract in doses fifty, five hundred, fifteen hundred and twenty-five hundred mg/kg body weight/day, respectively). As expected, the rat's body weight increased with time. Equally, no significant changes were recorded in body weight gain of control and treated rats (Table 2 and 3). However, the rats treated with

DRE did present mild diarrhoea at some point in the period of treatment, portentous of a laxative effect of the extract. The treatment with a hydro-alcoholic extract of *D. cinnabari*

for 28 days did not alter the biochemical profile of female and male S D rats, as shown in Tables 5 and 7, respectively.

Haematological analysis

In the same way, the haematological profile of female (Table 4) and male rats (Table 6) was not altered by treatment with DRE. In both biochemical and haematological studies, all values in control and treated groups were within the reference range for the species.

DISCUSSION

Herbal medicines are used worldwide for the treatment and prevention of various acute and chronic diseases and are gaining popularity in developing countries. Herbal medicines are often believed to be harmless because they are "natural" and are easily available and commonly used for self-medication without supervision. These medicines contain 7bioactive constituents with potential to cause adverse effects. [16, 17]. The results of the present study suggested that *D. cinnabari* is a relatively nontoxic plant. During 120 hours period of acute toxicity evaluation, rats were not associated with any mortalities and abnormalities in general conditions, behavior and growth, feed and water consumption of animals. Body weight changes are indicators of adverse side effects, as the animals that survive cannot lose more than 10% of the initial body weight [18]. Body weight gain and feed consumption levels were similar in both control and treated animals.

ALP, ALT, AST in tissue and blood are important marker enzymes which are used to assess the integrity of the cell membrane, cytosolic activity and cell death [19]. The extent of hepatocellular injury is assessed by the increased serum levels of ALP, ALT, and AST .The study results indicate that the value of marker enzymes was within normal permissible limits and same was observed in case of various hemaetological parameters.

CONCLUSION

In conclusion, the DRE was well tolerated, lack of mortality and neither produced overt signs of clinical toxicity (loss of hair, behavioral changes, impairments in feed intake and body weight gain), nor any signs of hepato-, nephro-, or haematotoxicity, also well supported by biochemical data. This acute toxicity study suggests that the extract of *D. cinnabari* resin is safe up to the dose of 2500mg/ kg b.w. especially when consumed by oral route. Further studies in repeated doses (sub-acute and chronic) must be performed to prove its safety.

REFERENCES

1 Dias, F D L , Takahashi C S , "Cytogenetic evaluation of aqueous extracts of the medicinal plants *Alpinia mutans* rose (Zingerberaceae) and *Pogostemum hyneanus* Benth (Labitae) on Wistar rats and *Allium cepa* (Liliaceae) root tip cells," Brazilian Journal of Genetics, 1994: 17(2):175–180 .

2 Hossan M S, Hanif A, Khan M, Bari S, Jahan R, Rahmatullah M. Ethnobotanical survey of the Tripura tribe of Bangladesh. Am Eurasian J Sustain Agric 2009; 3(2):253-61.

3 Ukwuani A N, Abubakar M G,Hassan S W , Agaie BM, "Toxicological studies of hydromethanolic leaves extract of Grewiacrenata," International Journal of Pharmaceutical Science and Drug Research 2012; 4:245–249.

4 Zhou Z.H. , Wang J.L. , Yang C.R. , Cochinchinenin--a new chalcone dimer from the Chinese dragon blood. Acta Pharm. Sin. 2001; 36:200–204.

5 Silva B M, Santos R P,Mendes L S, De Pinho P G, Valentão P ,Andrade P B ,Pereira J A , Carvalho M, *Dracaena draco* L. fruit: Phytochemical and antioxidant activity assessment, Food Research International, 2011; 44(11):2182-2189.

6 Xina N,Li Y J, Li Y, Dai R-J, Meng W-W, Chen Y, Schlappi M,Deng Y-L ,Dragon's Blood extract has antithrombotic properties, affecting platelet aggregation functions and anticoagulation activities, J of Ethnopharmacol, 2011: 135(2):510-514.

7 Li YS, Wang JX, Jia MM, Liu M, Li XJ, Tang HB. Dragon's blood inhibits chronic inflammatory and neuropathic pain responses by blocking the synthesis and release of substance P in rats. J Pharmacol Sci. 2012; 118(1):43-54.

8 Gupta, D., Bleakley, B., Gupta, R.K.; Dragon's blood botany chemistry and therapeutic uses; Journal of Ethnopharmacology, 2008; 115: 361–380.

9 O'Neill J, Bray H, Boardman P, Chan L. Antimalarial activity of *Brucea Javanica* fruit. J Pharmacol. 1985; 37:49–57.

10. OECD Guidelines for the testing of chemicals: 423; 2001. Acute oral toxicity- Acute Toxic Class Method

11. OECD Guidelines for the testing of chemicals: 407; 2008. Repeated dose 28- day oral toxicity study in rodents

12. Sathya M, Kokilavani R, Teepa KSA. Acute and subacute toxicity studies of ethanolic extract of Acalypha indica Linn in male Wistar albino rats. Asian J Pharm Clin Res 2012; 5:97-100.

13. Banerjee S, Chattopadhyay P, Ghosh A, Pathak M P , Singh S, Veer V Acute Dermal Irritation, Sensitization, and Acute Toxicity Studies of a Transdermal Patch for Prophylaxis Against (+) Anatoxin-A Poisoning International Journal of Toxicology 2013; 32(4):308-313.

14. Chanda S, Dave R, Kaneria M, and Shukla V Acute oral toxicity of *Polyalthia longifolia* var. pendula leaf extract in Wistar albino rats. Pharma Biol 2012: 50 (11):1408-1415.

15. Bigoniya P, Sahu T, Tiwari V. Hematological and biochemical effects of sub-chronic artesunate exposure in rats. Toxicol Rep 2015; 2:280-8.

16. Saad B, Azaizeh H, Abu-Hijleh G, Said O. Safety of traditional Arab herbal medicine. Evid Based Complement Alternat Med, 2006; 3:433–439.

17. Bent S, Ko R, Commonly used herbal medicines in the United States: A review. Am J Med, . 2004; 116:478–485.

18. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of d-methylphenidate and d, lmethylphenidate in Sprague-Dawley rats. Toxicology, 2002; 179:183–196.

19. Akanji MA, Olagoke OA, Oloyede OB. Effect of chronic consumption of meta-bisulphite on the integrity of the rat kidney cellular system. Toxicology, 1993; 81:173–179.