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STUDY OF TOXICITY PARAMETERS ON COMBINED HERBAL EXTRACT OF *ASYSTASIA GANGETICA, FICUS RECEMOSA* AND *MORUS INDICA* USING EXPERIMENTAL RATS.

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ABSTRACT

Aim of this study is to create a data on the safety and toxic levels of this combined extract preparation for further screenings and usages. Mostly single plant extract toxicity and safety study is being done, but required data for this combined extract preparation-OECD 425 guideline is followed to conduct toxicity effect for this study. This guideline was preferred to conduct the toxicity, due to 1) minimize the number of animals to estimate LD50 and confidential interval (CI). estimation. 2) also the guideline OECD 425 was concurrently under taken with revision of test guidelines 420 and 423 for oral toxicity testing. The ethanolic extract of *Asystasia gangetica (A- Acanthacae*a), *Ficus recemosa (F-Moraceae)* and *Morus indica (M-Moraceae)* evaluated for acute toxicity (*AFM*) in adult wistar rats. The ethanolic extract of AFM (*A+F+M)* was administered as 175(50+60+65)mg, 500(150+175+175)mg, 1500(450+525+525)mg and 5000(1500+1700+1750)mg. The first group animal is dosed a step below (~ly,-3.2 factor) the best preliminary estimation of LD 50 (<5000mg) the moribund (nearing to death stage) was observed and the dose was reduced to 3.2 factor level i.e., 5000mg/kg as combination as 175, 500, 1500, 5000, since the dosing in standard from 175mg/kg due to no estimation of the sub-standard lethality is available. The final results were statistically analyzed after **14days** and tabulated. The results revealed that no abnormalities in treated animals were found than the control animals.

KEYWORDS: Safety, toxicity, combined extract, OECD 425, *Asystasia gangetica, Ficus recemosa, Morus indica.*

1. INTRODUCTION

Herbal medicine is one of the most oldest form of medical treatment. From the beginning of human progress people are bound to nature in many ways. The traditional plants and natural products are being used for prevention, reduction and cure of diseases which was the approach of primitive healthcare system. The primary health care in most of these old civilized societies depends on herb based medicines.

In last part of twentieth century, western nations concluded the importance of herbal medicine as the one that possesses best health benefits with less adverse effects and countries like USA, UK, Australia and other European countries have preferred the medication. Herbal drugs are recently prepared mostly by environmental processes from plants and can be defined as preparations containing active constituents of medical importance.[1]

The traditional systems of medicine re-established in all over the world in light of present technological aspects. Promotion in different areas of herbal research starting from extraction procedures to isolation & identification techniques, design & usage of bioassay for capability testing, dosage form design as well as study of pharmacokinetic, pharmacodynamic, toxicological and pharmacological mode of action.

Asystasia gangetica* (L).** T. Anders the plant juice is used in the treatment of swelling, rheumatism. It also acts as anthelmintic, antipruritic, galactogogue and intestinal astringent. The leaves of the plant are reported to posses anti-asthmatic activity.[21] ***Ficus recemosa linn the plant extract is used and reported as anti diuretic, anti tussive, anti diarrhea, anti ulcer etc., and ***Morus indica*** leaf extract is reported as analgesic, anti helmentic, anti bacterial etc. The objective of this study is to investigate the safety and toxicity of these extracts as combined preparation for further screenings and usages.[16,17,18]

Primary considerations

1) All about the plant profiles are studied and the Phytochemical ingredients are identified generally and it contains Tri terpenoids, Flavonoids, Glycosides, Steroids, alkaloids, Saponins, Carbohydrates, Proteins and Tannins.[19]
2) The in-vitro observation studies are performed and the anti oxidant proportion were analyzed to the plant extracts and the results were arrayed.[22]
3) From the in-vitro study the anticipated uses of the substance is surveyed by various articles. This relevant study helps to know the protection of humans and the environment

and it will help to select an appropriate dose of the drug to start the treatment for the study.[22]

Maintain conditions applied

1) The intervals between dosing is determined by onset, duration and severity of toxic signs (normally 48 hrs), from this the approximate LD 50 values calculated.[8]
2) The first animal is dosed a step below (-3.2 factor) the best preliminary estimation of LD 50 (<5000mg) the abnormalism was observed and the dose was reduced to 3.2 factor level ie, as combination as 175, 500, 1500, 5000, since the dosing is standard from 175mg/kg due to no estimation of the sub-standard lethality.
3) This procedure is known as **up and down procedure (UDP)** for the determination of acute toxicities for chemicals.[18]

OBSERVATION

1) All the animals were observed 30mints once, weekly during first 4hrs and periodically for 24 hrs then upto **14days** no toxic signs were observed all the observed symptoms are recorded individually such as weight (weekly) skin, fur, eyes, mucous membrane, respiratory, ANS, CNS, Somatomotor activities. Behavioral activities of animal tremor, convulsion, salivation, diarrhoea, lethargy, sleep, coma and no death was found.[14]
2) Animal weight was recorded on weekly basis.
3) At the end of the toxicity study test all the animals were weighted and authorized for euthanasia. All the pathological changes were recorded for each animals and compared with normal control tissue.
4) All the animal were kept under fasting over night with water ad-libitum prior to dosing, then weighed for test drug dose calculation and administered as per body weight. The animals even after drug administration food was withhold for further 3.5 hrs then the animals were fed.[11,12]

2. MATERIALS AND METHODS

Drugs and chemicals

Streptozotocin purchased from M/S. Otto chemicals-Mumbai. Glibenclamide was obtained from AASSK Pharmaceuticals-Chennai. Hb kit from span diagnosis-Kerala. **SD** *CHECK (*blood glucose test strip-GOLD) from SD biosensor, Germany-Tirupathi. Animal feed from Hindustan limited. All other chemicals used in the study were of analytical grade procure from BROSS chemicals Tirupathi.

Ethical Clearance

The study was approved by the Animal Ethics Committee, Sri Padmavathi school Of Pharmacy, Affiliated to JNTUA University. Anantapuram.

Plant materials

Collection and Authentification

The of *Asystasia gangitica*(L.). T. Anders. Had been collected from marudhamalai hills of Coimbatore district, during the month of may and June 2012 (senthilkumar, et al., 2006).the plant was identified and authenticated by Dr. G.V.S Moorthy, joint director. Botanical survey of India, Tamilnadu agricultural university (TNAU), Coimbatore, India and the voucher specimen has been given the code BSI/SC/5/23/07-08/Tech-290 dated 05.06.2007. *Ficus recemosa* (19-08-2010) and *Morus indica* (03-04-2009) were authenticated by DR. K. Madavachetty, Asst. professor, Dept Of Botany, Sri Venkateswara University, Tirupathi (AP-India) and were collected from Yogi mallavaram (Tirupathi- chittoor district A.P) and Karvettinagaram (Puttur- chittoor district A.P) respectively.

Preparation of the Extract

The fresh leaves of the plants were collected and air dried under shade at room temperature. The leaves were individually powdered mechanically and stored in air tight container for experimental purposes separately. Maceration technique was followed for extraction of leaves. 70% ethanol was used as solvent. About 600gm of powder was mixed with 1700ml of solvent and kept on mechanical shaker for 4hrs and filtered. The filtered marc obtained was again added with solvent and the procedure was repeated. The contents obtained after shaking was filter through muslin cloth and the filtrate was concentrated under reduced pressure and control temperature to yield a dark gummy solid. The extract was preserved in a refrigerator at 4^{o}C. The percentage yield for all the extracts were calculated and given in the table ie.,

% yield = {weight of extract produced/weight of leaf powder taken} x 100

Phyto Chemical Screening

Chemical tests were carried out using the extract of *Asystasia gangitica*, *Ficus recemosa* and *Morus indica* for the presence of phyto chemical constituents.[19]

Experimental Animal species selection

The adult healthy wistar strain male and female rats were used for this studies, generally female rats are slightly more sensitive these animals are normaly 10 weeeks of age and 150-160gm in weight.

Housing and feeding

Animals house with 22+3^{0}c, Relative humidity approximately 50% was maintained, Artificial lighting and 12hrs light and dark was maintained and Animal pellets with adequate water was supplied.[2]

Animal preparation

The randomly selected animals were marked for individual identifications. All the animals were individually kept in their cases for 5days before dosing to allow for acclimatization to the laboratory conditions.

Experimental animals

N=5 and n=5

Group 1: Normal control (10ml Normal saline/kg)

Group 2: 175(50+60+65),

Group 3: 500(150+175+175),

Group 4: 1500(450+525+525),

Group 5: 5000(1500+1700+1750).

Dose preparation

The plant extracts were thoroughly mixed with normal saline and 1.5ml to 2.0 ml/100g of animal was preferred due to non toxic and sterilized vehicle, the dose was prepared freshly and administered to maintain the stability of plant extract.

Dose administration

1) The test drug was administered in a single dose by gavages using stomach tube.
2) All the animal were kept under fasting over night with water ad-libitum prior to dosing, then weighed for test drug dose calculation and administered as per body weight. The animals even after drug administration food was withhold for further 3.5 hrs then the animals were fed.[11,12]

LABORATORY OBSERVATIONS

A) General Observations and Animal Behavior

The rats were keenly observed constantly during first 30 minutes after dosing, intermittently next 24 hours and then daily, thereafter, for 14 days. All observed findings were scientifically documented for individual rat such as Abdominal gripping or writhing, Body weight, Circling motion (motor behavior during cognitive tasks-assessed by an Actophotometer), Colpectasia, Coma, Convulsion, Diarrhea, Eyes (Pupil size, Lacrimal secretion, Right reflex and corneal reflex-PLRC), Lethargy, Micturation, Mortality, Mucous Membrane, Pilomotor erection, Priaprism, Robichaud test, Rectal temperature, Salivation, Skin & Fur, Sleep, Tail erection or Straub response, Tail lashing, Urination, walking behavior All the findings are based on pharmacological screening ratings.

B) Hematological parameters

RBC, WBC, DIFFERENTIAL LEUCOCYTE COUNTS (Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Platelets, Bleeding time, Clotting time, ESR.

3.0 RESULTS

a) Percentage yield of ethanolic extracts of *Asystasia gangetica*, *Ficus recemosa* and *Morus indica* leaves.

Table no: 1 Percentage yield of *Asystasia gangetica, Ficus recemosa,* and *Morus indica* leaves.

S.No	Extract	solvent	% yield w/w
1	*Asystasia gangetica*	70% Ethanol	22.50
2	*Ficus recemosa*	70% Ethanol	15.85
3	*Morus indica*	70% Ethanol	20.24

b) The extract was taken for Phytochemical screening tests and found the ingredients present in that as tabulated.

Table no: 2 Phytochemical screening of AGLE, FRLE & MILE.

S.NO	CONSTITUENTS	AGLE	FRLE	MILE
1.	Tri terpenoids	+	+	+
2.	Flavonoids	+++	-	+
3.	Glycosides	-	+	++
4.	Steroids	+	+	-
5.	Alkaloids	+	+++	+

6.	Saponins	+	-	-
7.	Carbohydrates	+	+	+
8.	Proteins	+	+	+
9.	Tannins	+	+	+

(+) Presence of constituents (-) Absence of constituents.

Table no: 3 Physiological and general parameters effects at 175, 500, 1500 and 5000mg/kg body wt of Poly Herbal Preparation on normal rats Table conti----- conti-----

S.No	General Parameters	Normal Control*	0th day				7th day(mg/kg)				14th day(mg/kg)			
			175(mg/kg)	500(mg/kg)	1500(mg/kg)	5000(mg/kg)	175(mg/kg)	500(mg/kg)	1500(mg/kg)	5000(mg/kg)	175(mg/kg)	500(mg/kg)	1500(mg/kg)	5000(mg/kg)
1)	Locomotor action	N	N	N	N	N	N	N	N	N	N	N	N	Mc
2)	Colpectasia	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
3)	Coma	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
4)	Convulsion	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
5)	Diarrhea	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Pcs
6)	Eyes(PLRC)	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc
7)	Lethargy	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
8)	Micturation	N	N	N	N	N	N	N	N	N	N	N	N	N
9)	Mortality	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
10)	Mucous Membrane	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Cp
11)	Pilomotor erection	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc
12)	Priaprism	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc

Table no: 3 Physiological and general parameters effects at 175, 500, 1500 and 5000mg/kg body wt of Poly Herbal Preparation on normal rats.

S.No	General Parameters	Normal Control*	0th day(mg/kg)				7th day(mg/kg)				14th day(mg/kg)			
			175	500	1500	5000	175	500	1500	5000	175	500	1500	5000
14	Rectal temperature	N	N	N	N	N	N	N	N	N	N	N	N	N
15	Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N
16	Skin & Fur	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc
17	Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N
18	Straub response	0	0	0	0	0	0	0	0	0	0	0	0	0
19	Tail lash	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc
20	walking	N	N	N	N	N	N	N	N	N	N	N	N	N

N – Normal**: MC**-Moderate change: **Na**- Not Available: **NC**- No change**: Ab**-Absent: *- In all doses and days**: PCS**-Pale colored stooling**: CP** - Colored papillae patches: **0** –No response.

Table no: 4 Effect of PHP on Central Nervous System.

S.No	General Parametes	Normal Control*	0th day(mg/kg)				7th day(mg/kg)				14th day(mg/kg)			
			175	500	1500	5000	175	500	1500	5000	175	500	1500	5000
2	Anesthesia	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
3	Ataxia Rating	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
5	Pinna Reflex	0	0	0	0	0	0	0	0	0	0	0	0	0
6	motor activity	0	0	0	0	0	0	0	0	0	0	0	0	0
8	muscle grip	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc	Nc
9	Paralysis forelegs	0	0	0	0	0	0	0	0	0	0	0	0	0
	Paralysis hind legs	0	0	0	0	0	0	0	0	0	0	0	0	0
	head	N	N	N	N	N	N	N	N	N	N	N	N	N
10	Respira tory Rate	0	0	0	0	0	0	0	0	0	0	0	0	0

N – Normal**: MC**-Moderate change: **Na**- Not Available: **NC**- No change**: Ab**-Absent: *- In all doses and days**: PCS**-Pale colored stooling**: CP** - Colored papillae patches: **0** –No response.

Table no: 5 Hematological parameters.

S. No	parameters	Normal	0th day(mg/kg of test drug)				7th day(mg/kg of test drug)				14th day(mg/kg of test drug)			
			175	500	1500	5000	175	500	1500	5000	175	500	1500	5000
1.	**Body weight(gm)**	180.60 ± 1.56	178.50 ±1. 86	187.00 ±1.23	191.30 ± 2.03	187.90 ± 2.81	182.34 ± 2.09	194.56 ±1.93	193.43 ± 1.74	191.39 ± 1.58	186.80 ± 1.83	202.45 ± 1.94	199.50 ± 2.09	195.37 ± 2.01
2.	**RBC (10^6/mm^3)**	8.25 ±1.29[a]	8.47 ±1.96 [a]	7.94 ±1.76 [a]	9.02 ±2.09 [a]	6.91 ±0.97 [a]	8.55 ±0.86 [a]	7.97 ±1.65 [a]	9.25 ±1.23 [a]	6.65 ±1.45 [a]	8.53 ±0.98 [a]	8.00 ±1.45 [a]	9.31 ±1.35 [a]	7.25 ± 1.65 [a]
3.	**WBC (10^3 /mm^3)**	8.34± 1.47[a]	8.87± 2.67[a]	7.87± 2.89[a]	9.13± 0.29[a]	8.68± 2.68[a]	8.99± 3.48[a]	8.01± 0.57[a]	9.37± 1.86[a]	8.91± 2.83[a]	9.05± 1.28[a]	8.35± 1.78[a]	9.56± 0.87[a]	8.75± 1.65[a]
4.	**DLC(%):** Neutrophils	40.09 ±0.94[b]	46.91 ±1.37[a]	39.76 ±1.75 [a]	42.48 ±1.46 [b]	37.97 ±1.30 [a]	47.35 ±3.09 [a]	43.27 ±1.47 [b]	44.47 ±1.86 [a]	41.16 ±1.89 [a]	46.37 ±1.27 [a]	45.72 ±1.50 [a]	47.71 ±1.30 [b]	45.51 ±1.20 [b]
5.	Lymphocytes	79.98 ±0.25[a]	77.93 ±0.76 [a]	83.95 ±0.37 [a]	81.74 ±0.75 [a]	78.49 ±1.87 [a]	80.01 ±1.34 [a]	84.93 ±1.20 [a]	80.47 ±1.63[b]	76.48 ±1.98[b]	82.57 ±1.29 [a]	85.93 ±1.36 [a]	81.97 ±1.47 [a]	79.01 ±1.26 [a]
6.	Monocytes	3.47 ±1.47 [a]	3.87 ±1.34 [a]	3.08 ±1.73 [a]	3.75 ±0.89 [a]	2.95 ±0.82 [a]	3.91 ±0.19 [a]	3.18 ±1.01 [a]	3.81 ±1.04 [a]	3.27 ±1.05 [a]	4.05 ±1.63 [a]	3.26 ±2.17	3.69 ±1.76[b]	3.18 ±1.09[b]
7.	Eosinophils	0.91 ±1.36[a]	1.09 ±1.75 [a]	0.11 ±0.95 [a]	0.97 ±0.46 [a]	1.92 ±2.45 [a]	0.73 ±2.86 [a]	0.08 ±1.04 [a]	1.06 ±3.01 [a]	2.03 ±3.21 [a]	0.61 ±1.20 [a]	0.21 ±1.04 [a]	0.99 ±2.04 [a]	2.35 ±1.28 [a]
8.	Basophils	0.71 ±0.19 [a]	0.91 ±1.63 [a]	0.31 ±1.73 [a]	0.84 ±1.87 [a]	0.67 ±1.54 [a]	0.85 ±1.97 [a]	0.27 ±0.36 [a]	0.72 ±1.85 [a]	0.71 ±1.22[b]	0.73 ±1.77 [a]	0.37 ±1.46[b]	0.91 ±2.90[b]	0.93 ±1.37[b]
9.	**PLATELETS (10^3/uL)**	759.01± 2.09 [a]	821.19 ±2.17 [a]	679.27 ±1.53 [a]	833.38 ±1.23 [a]	791.17 ±1.76 [a]	834.41 ±1.82 [a]	701.51 ±0.91 [a]	893.23 ±0.94 [a]	803.65 ±1.00 [a]	855.09 ±1.74 [a]	725.89 ±2.95 [a]	871.56 ±3.12[b]	842.62 ±3.02[b]

Data are expressed as mean ± S.E.M; (n=6). Values are statistically significant at P< (0.01^a-0.05^b) compared with control.

Table no: 6 Hematological parameters.

Treatment		HB%	ESR	FBG	PPG	Bleeding time (seconds)	Clotting time (minutes)
Normal control		13.01± 0.09[a]	13.93±1.93[a]	105.72 ± 0.08[a]	121.76± 0.08[a]	30.55 ± 0.11[b]	4.20 ± 0.34[b]
0th day (mg/kg)	175	11.65± 0.67[a]	9.23±2.95[a]	101.00 ± 0.17[a]	120.64± 0.08[a]	31.23± 0.10[b]	3.52 ± 0.64[b]
	500	10.87± 0.54[a]	10.93±1.39[a]	100.88± 0.05[a]	119.46± 0.08[a]	33.45± 0.19[b]	3.57 ± 0.98[b]
	1500	10.10± 0.65[a]	10.72±1.02[a]	107.53± 0.20[a]	127.56± 0.08[a]	32.45± 0.97[b]	4.19 ± 1.51[b]
	5000	12.15± 0.09[a]	14.83±1.30[a]	96.06± 0.98[a]	117.96± 0.08[a]	33.36± 1.78[b]	4.51 ± 0.96[b]
7th day(mg/kg)	175	12.09± 0.06[a]	9.64±2.47[a]	97.75± 0.09[a]	116.09± 0.08[a]	32.59± 1.45[b]	3.57 ± 1.21[b]
	500	11.06± 0.14[a]	11.63±2.18[a]	97.09± 0.33[a]	119.87± 0.08[a]	34.57± 1.34[b]	4.03 ± 0.43[b]
	1500	11.04± 0.25[a]	10.42±1.98[b]	105.65± 0.67[a]	125.06± 0.08[a]	33.45± 1.56[b]	4.25 ± 0.67[b]
	5000	12.03± 1.36[a]	13.53±0.93[b]	95.45± 0.86[a]	117.97± 0.08[a]	35.32± 1.67[b]	4.57 ± 1.24[b]
14th day(mg/kg) 14th day(mg/kg)	175	12.11± 1.36[a]	9.93±0.84[a]	93.23± 0.60[a] **(-8.33%)**	112.93± 0.08[a] **(-6.83%)**	34.12± 1.89[b]	4.02 ± 0.14[b]
	500	11.16± 0.76[a]	11.03±0.67[b]	95.98± 0.09[a] **(-5.11%)**	116.99± 0.08[a] **(-2,11%)**	36.35± 0.00[b]	4.09 ± 0.10[b]
	1500	11.12± 0.89[a]	10.01±0.86[b]	101.09± 0.11[a] **(-6.37)**	123.66± 0.08[a] **(-3.15)**	35.17± 0.02[b]	4.32 ± 1.17[b]
	5000	13.15± 1.89[a]	13.00±0.28[b]	93.86± 0.56[a] **(-2.34%)**	**117.65± 0.08[a] (-0.26%)**	35.24± 0.11[b]	4.04 ± 1.11[b]

Data are expressed as mean ± S.E.M; (n=6). Values are statistically significant at P< (0.01[a] 0.05[b]) compared with control.

4.0 CONCLUSION

From this study the conclusion clearly indicates that in the gradual incremental of dose 1500mg and 5000mg Showed some statistical irrelevant (pale colored stooling, FBG, PPG and Eosinophil changes but no mortality evidences. The LD50 value lies below 5000mg. and the narrow efficassy seems to be between 175mg and 500mg, so to avoid dose dumping 175mg as a combined drug was suggested for further treatment more over 175mg/kg also showed effectiveness in the better increase of body weight, RBC, WBC, in controlling of FBG and PPG so furtherly the dose was adjusted as AFM(*A+F+M)* was administered as 200(50+75+75)mg by UP AND DOWN method of dosage calculation.

5.0 REFERENES

1. Vipul Gujrati, Nilesh Patel, Venkat N Rao, K Nandakumar, TS Gouda, Md. Shalam, SM Shanta Kumar. Hepatoprotective activity of alcoholic and aqueous extracts of leaves of *Tylophora indica* (Linn.) in rats. indin journal of pharmacology, 2007; 39: 43-47.

2. M. Shankar, N.L. Gowrishankar, C. David Raj, MD. Ansar, P. Pranathi, G. Raju, A. Shravan kumar, A. Uday and V. Usha. Screening of Methanolic Extract of *Eugenia Jambolana* Leaves for its Hepatoprotective Activity in Carbon Tetrachloride Induced Rats. International Journal of Applied Research in Natural Products., 2012; 5(2): 14-18.
3. V. kishor kumar, K.G. lalitha, Acute oral toxicity studies of *anacyclus pyrethrum* dc root in albino rats. International Journal of Pharmacy and Pharmaceutical Sciences, 2013; 5(4): 675-78.
4. Vishnu S. Neharkar, Dr. Sunil Kshirsagar, Dr. Ramdas Pandhare Acute toxicity study of *Hygrophila auriculata* leaves methanolic extract in albino rats. Journal of Pharmaceutical, Chemical and Biological Sciences., 2015; 3(3): 388-95.
5. Jagir R. Patel, Moses Samuel Rajan, Shenoy Ashok, Shabaraya A. R. Protective Effect of *Uvaria narum*. Leaves on Carbon Tetrachloride induced Hepatotoxicity in Rats. Journal of Applied Pharmaceutical Science, 2013; 3(07): 164-68.
6. Umana, Uduak E., Timbuak, J.A., Musa, S.A, Samuel Asala, Joseph Hambolu and Anuka J.A. Acute and Chronic Hepatotoxicity and Nephrotoxicity Study of Orally Administered chloroform extract of *Carica papaya* Seeds in Adult Wistar Rats. International Journal of Scientific and Research Publications, 2013; 3(4): 1-8.
7. Vipul Gujrati, Nilesh Patel, Venkat N Rao, K Nandakumar, TS Gouda, Md. Shalam, SM Shanta. Kumar Hepatoprotective activity of alcoholic and aqueous extracts of leaves of *Tylophora indica* (Linn.) in rats. Ref cross citation., 2007; 39(1): 43-47.
8. Jegathambigai R Naidu, Rusli Ismail1 and Sreenivasan Sasidharan. Acute Oral Toxicity and Brine Shrimp Lethality of Methanol Extract of *Mentha Spicata* L (Lamiaceae). Tropical Journal of Pharmaceutical Research January., 2014; 13(1): 101-0.
9. Feroz Ahmad, Nahida, Tabassum and Majid Ahmad. Preliminary phytochemical, acute oral toxicity and antihepatotoxic study of roots of *Paeonia officinalis* Linn. Asian Pacific Journal of Tropical Biomedicine., 2013; 3(1): 64-68.
10. Khalid Algariri, Justin Atangwho, Kuong Yow Meng, Mohd Zaini Asmawi, Amirin Sadikun and Vikneswaran Murugaiyah. Antihyperglycaemic and Toxicological Evaluations of Extract and Fractions of *Gynura procumbens* Leaves. Tropical Life Sciences Research., 2014; 25(1): 75–93.
11. G.T. Volpato, M.P. Calderon, S. Sinzato, K.E. Campos, M.V.C. Rudge, D.C. Damasceno. Effect of *Morus nigra* aqueous extract treatment on the maternal–fetal outcome, Oxidative stress status and lipid profile of streptozotocin-induced diabetic rats Journal of Ethnopharmacology., 2011; 138: 691-96.

12. Prasanth Kumar. M, Suba. V, Ramireddy. B and Srinivas Babu. P. Acute and Sub-Acute (28-Day) Oral Toxicity Studies of Ethanolic Extract of *Celtis Timorensis* Leaves in Rodents. Global Journal of Medical Research: B Pharma, Drug Discovery, Toxicology and Medicine {Type: Double Blind Peer Reviewed International Research Journal Publisher: Global Journals Inc. (USA)., 2014; 14(3)/Version 1.0, 1-8.
13. B. Gandhare1, S. Kavimani, and B. Rajkapoor. Acute and Subacute Toxicity Study of Methanolic Extract of *ceiba pentandra*(Linn.) Gaertn. on Rats. JOURNAL OF SCIENTIFIC RESEARCH., 2013; 5(2): 315-24.
14. Panunto W, Jaijoy K, Lerdvuthisopon N, Lertprasertsuke N, Jiruntanat N, Soonthornchareonnon N and Sireeratawong S. Acute and chronic toxicity studies of the water extract from dried fruits of *Terminalia chebula* Rezt. in rats. International Journal of Applied Research in Natural Products, 2011; 3(4): 36-43.
15. Khalid Algariri, Item Justin Atangwho, Kuong Yow Meng, Mohd Zaini Asmawi, Amirin Sadikun and Vikneswaran Murugaiyah. Antihyperglycaemic and Toxicological Evaluations of Extract and Fractions of Gynura procumbens Leaves. Tropical Life Sciences Research, 2014; 25(1): 75–93.
16. www.oecdilibrary.org test no 420 acute oral toxicity fixed dose procedure.
17. www.oecdilibrary.org test no 423 acute oral toxicity fixed dose procedure.
18. www.oecdilibrary.org test no 425acute oral toxicity fixed dose procedure.
19. Trease, G.E. and Evans, M.C. Text book of Pharmacognosy. 12th, Bailier, tindall, London, 2002; 343-82.
20. Kiritikar, K.R. and Basu, B.D. Indian medicinal plants. International book distributors. Dehradun, 3: 1891-92.
21. Senthilkumar, M., Gurumoorthi, P. and Janardhanan, K. Some medicinal plants used by Irular, the tribal people of Marudhamalai hills, Coimbatore, Tamil nadu. Natural Product Radients, 5: 382-88.
22. Lamaison, J.L., Pettijean-freytet, C., Carnet, A.P. and Carnat, A. Activites antiradicalaires in vitro d'huiles essentielles. plants medicines et phototherapy, 1988; 22: 231-234.
23. Larson ra. the antioxidants of higher plants phyto chemistry., 1988; 27(4): 969-978.