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A RESEARCH ON TOXICITY STUDIES ON COMBINATION EXTRACTS OF THREE PLANTS- *TERMINALIA ARJUNA*, *CHRYSANTHEMUM INDICUM* AND *MORINGA OLEIFERA.*

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ABSTRACT

Natural medicine, especially from herbs, is the source for the research of various novel medicinal compounds. Drugs from herbal origin must be ensured as safe before used as medicine. Objective: The present work focused to study the toxicity effects of ethanolic and aqueous extracts of Bark of *Terminalia arjuna*, flowers of *Chrysanthemum indicum*, leaves of *Moringa oleifera*. Methods: Sub-acute toxicity study of aqueous and ethanolic extracts were conducted on Wistar albino rats according to the protocols described in OECD guidelines 407 [OECD, 2007]. All the animals were observed twice daily for mortality and morbidity. The clinical observation included changes in mucus membrane, eyes, fur, skin, and autonomic activity such as piloerection, changes in pupil size, lacrimation and unusual breathing pattern. Results: No mortalities were recorded in rats over the period of 30 days of treatment with both the CTAE (combination of three aqueous extracts) and CTEE (combination of three ethanolic extracts) at the doses of 125, 250 and 500 mg/kg, body weight, through oral route. None of the rats after administration of CTAE and CTEE at the doses of 125, 250 and 500 mg/kg body weight, showed any obvious morbidity or clinical symptoms of toxicity such as changes in the skin and fur, eyes, respiratory rate, autonomic (salivation, perspiration and piloerection), and stereotype activities throughout the experimental period of 30 days. Conclusion: There were no clinical signs of toxicity observed for the normal control group. As there was no mortality recorded for all the doses, the LD50 value was assumed to be greater than the limit test dose of 2000 mg/kg, body weight.Hence 125, 250 and 500 mg/kg, oral doses of both CTAE as well as CTEE were selected for further study.

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INTRODUCTION

Toxicity studies are important for selection of doses and new drug development processes. Numerous emergent countries are using herbs and herbal products for their health care needs. As per the literature survey too many allopathic medicines are derived from herbal sources. Ayurvedic medicine is still practiced in the world where approximately 85% of the Indian population uses herbal formulations for the cure of various diseases. On the other hand, the use of plants by tribal people for curing diseases without knowing its adverse effects may cause health complications in later stages [Cock IE et.al.]. There is a lack of scientific knowledge on the safety and efficacy of herbal drugs on the increase in a number of its users which has raised concerns regarding toxicity. Hence by considering the facts there is a need to calculate the safe and effective dose of the medicinal plants to enhance the use of herbal medicines for treatment of disease [Mohamed EAH].

MATERIAL AND METHODOLOGY

Chemicals and reagents

Ethanol 99.9% was procured from LOBA Chemicals, Mumbai. Ethylene di-aminetetraacetic acid (EDTA) was procured from Thermo Fisher Sci- entific India Pvt. Ltd., (Mumbai, India). All the solvents used were of high purity and HPLC grade. All other chemicals and reagents used in the whole study were of analytical grade.

Collection and authentication of plant materials

The Bark of *Terminalia arjuna*, flowers of Chrysanthemum indicum and leaves of Moringa oleifera were collected locally in the Ahmednagar district. The plant materials were then authenticated from College of agricultural science Loni, and was assigned with a Voucher no. ABM/Tech.II/2020/12 and specimen no. FH-01.

Figure: 4.2.1 Bark of *Terminalia arjuna.*

Figure 4.2.2 : Leaves of *Moringa oleifera.*

Figure: 4.2.3 Flowers of *Chrysanthemum indicum.*

Preparation of extracts

The collected plant material (Bark of *Terminalia arjuna*, flowers of Chrysanthemum *indicum* and leaves of *Moringa oleifera)* (500 g) each was gently washed by using distilled water to remove the impurities. The collected materials were shade dried in the laboratory under room temperature (24 ± 2 °C) for 3–4 weeks. After complete drying, the dried plant material was pulverized by using a mechanical grinder followed by sieving to obtain a coarse powder. The powdered plant material was then extracted with distilled water and ethanol (99.9%) using reflux technique separately. Extracts were concentrated by vacuum distillation and then dried in open air to produce the respective extracts. The crude aqueous and ethanol extracts obtained were stored at 4°C before analysis. The percentage yield of the extract was calculated by the following formula.

Percentage yield = (Weight of dry crude extract obtained / weight of plant material before extraction) X 100

The weight in grams was used to calculate the percentage yield. The calculated percentage yield was as follows;

No.	Extract	Percentage yield
1	Aqueous extract of Bark of *Terminalia arjuna*	7.24%
2	Aqueous extract flowers of *Chrysanthemum indicum*	6.34%
3	Aqueous extract leaves of *Moringa oleifera*	4.48%
4	Ethanol extract of Bark of *Terminalia arjuna*	8.54%
5	Ethanol extract flowers of *Chrysanthemum indicum*	5.35%
6	Ethanol extract leaves of *Moringa oleifera*	7.78%

PHYTOCHEMICAL SCREENING (Kokate CK et.al.)

The Phytochemical screening was done by the standard procedure as depicted in Table (Kokate CK *et.al*. 1994, Mondal S *et.al.* 2017).

Chemical constituents	Chemical test
Proteins	Biuret test
Carbohydrates	Molisch test
	Fehling's test
Alkaloids	Dragendorff's test
	Mayer's test
Steroids	Salkowski test
	Liebermann-burchard test
Triterpene	Vanillin-sulphuric acid test
Tannins	Ferric chloride test
	Dilute nitric acid test
Glycosides	Keller-killani test
Flavonoids	Shinoda test
	Lead acetate test
Saponins	Foam formation test
Amino acids	Ninhydrin test

ANIMALS USED IN STUDY

Adult healthy male and female Wistar albino rats with body weight 150–200 gm and 120–150 gm respectively were used to evaluate acute and sub-acute toxicity studies. The selected animals were kept in CPCSEA, New Delhi approved animal house under standard laboratory conditions. While Wistar albino rats weighing in between 120-150 gm and Swiss albino mice weighing in between 20 to 30 gm were selected for antihyperlipidemic activity.

The animal had free access to water and food ad libitum. The feed used was composed of crude proteins 16%, crude fats 3.8%, crude fibers 2%, amino acids, vitamins and minerals. The animals were kept in light-dark condition (12/12 h light/dark), temperature (22 ± 2°C) and humidity (55%). The Institutional Animal Ethics Committee has been sanctioned research project with vide certificate no. 1697/PO/Re/S/13/CPCSEA/2020/07.

Male and female rats were housed separately while maintaining standard condition. The animals were housed for at least one week in the laboratory experimental room prior to testing. Experiments were performed according to the guide for the care and use of laboratory animals.

Acute toxicity study

The acute toxicity studies were conducted over Wistar albino rats as per Organization for Economic Cooperation and Development (OECD) guidelines 423 [OECD, 2001] with a few minor modifications. Wistar rats of both male and female were used for the toxicity study. The selected male and female rats were then assigned to normal control and treatment groups. The test group rats received a combination of the aqueous and Ethanolic extracts of selected plants at the doses of 1000, 1500, and 2000 mg/kg, respectively. The aqueous extracts solution was prepared by dissolving extract in distilled water while Ethanolic extract was dissolved in tween 20 solution (1%, *v*/v)

The toxicity studies were performed for two extracts aqueous and ethanolic respectively. For the aqueous extract the control group was administered with distilled water while for the Ethanolic extract control group rats were given only tween 20 solution (1%, v/v) as vehicle. All rats were weighed, marked for identification, and fasted overnight but were allowed free access to water. After administration of dose, the animals were further fasted for 4 h and observations were recorded continuously for each individual rat in their respective groups during the first 4 h and then 24 h after drug treatment for any mortality and abnormal changes.

All the animals were then observed twice daily for a period of 14 days to find out any toxic effect viz., food and water intake. Acute toxicity studies were performed to provide information on short-term toxicity level of the test extracts. This toxicity study helps in the selection of doses for the repeated oral toxicity study.

Table no. 2.6.1: Acute toxicity study for CTAE (Combination of Three Aqueous Extracts).

Group No.	Treatment	Oral dose in mg/kg
I	Normal control (Distilled water)	-
II	CTAE	1000
III	CTAE	1500
IV	CTAE	2000

Table no. 2.6.2: Acute toxicity study for CTEE (Combination of Three Ethanolic Extracts).

Group No.	Treatment	Oral dose in mg/kg
I	Normal control (1%, v/v, tween 20)	-
II	CTEE	1000
III	CTEE	1500
IV	CTEE	2000

Sub-acute toxicity studies

Sub-acute toxicity studies of aqueous and Ethanolic extracts were conducted on Wistar albino rats according to the protocols described in OECD guidelines 407 [OECD, 2007].

Experimental grouping and dosing regimen

Wistar albino rats having average body weight between 150 and 200 g for males and 120–150 g for females were chosen for the study. They were assigned into eight groups (Six Animals in each group) based on body weight. Grouping of these animals were done such that the average body weight variation of the rats does not exceed ±25% of the mean body weight of each sex.

Sub-acute toxicity studies For CTAE

The animals in Group I served as a normal control group and received distilled water as vehicles. The animals of Group II, Group III and Group IV received mixture of combination of three aqueous extracts (CTAE) which includes Bark of *Terminalia arjuna*, flowers of *Chrysanthemum indicum* and leaves of *Moringa oleifera* at the doses of 125, 250 and 500 mg/kg body weight, respectively, for 30 days. The dosing volume was 10 mL/kg and was orally administered once daily through oral route. All animals were allowed for free access to food and water throughout the study.

Table no. 2.7.2.1 Sub-acute toxicity studies for CTAE.

Group No.	Treatment	Oral dose in mg/kg
I	Normal control (Distilled water)	-
II	CTAE	125
III	CTAE	250
IV	CTAE	500

Sub-acute toxicity studies For CTEE

The animals in Group I served as a normal control group receiving tween 20 solutions (1%, v/v) in distilled water as vehicles. The animals of Group II, Group III and Group IV received mixture of combination of three ethanolic extracts (CTEE) which include Bark of *Terminalia arjuna*, flowers of *Chrysanthemum indicum* and leaves of *Moringa oleifera* at the doses of 125, 250 and 500 mg/kg per body weight, which was suspended in 1%, v/v, tween 20 solution, respectively, for 30 days. The dosing volume was 10 mL/kg and was orally administered once daily through oral route. All animals were allowed for free access to food and water throughout the study.

Table no. 2.7.3.1 Sub-acute toxicity studies for CTAE.

Group No.	Treatment	Oral dose in mg/kg
I	Normal control (1%, v/v, tween 20)	-
II	CTEE	125
III	CTEE	250
IV	CTEE	500

Clinical observations and survival

All the animals were observed twice daily for mortality and morbidity. The clinical observation included changes in mucus membrane, eyes, fur, skin, and autonomic activity such as piloerection, changes in pupil size, lacrimation and unusual breathing pattern.

Changes in gait and posture were also monitored along with stereotype activities such as excessive grooming, repetitive circling etc. The period of observation was one week prior to administration of the test drug till scheduled necropsy.

Body weight

The body weights of all selected animals were recorded weekly (5 days interval) during the complete duration of the study. The body weights were also recorded prior to testing and terminally (after fasting) prior to necropsy.

Haematological analysis

Haematological profile of the blood samples kept in sterile tubes containing anticoagulants was analysed using an automatic haematological analyser. The haematological parameters investigated were as follows: haemoglobin, total white blood cell (WBC) count, total red blood cell (RBC) count, haematocrit (HCT), platelets (PLT), mean corpuscular volume (MCV), mean corpuscu- lar haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), differential leucocyte count (neutrophils, lymphocytes, eosinophil, monocytes).

Biochemical analysis

Blood serum was obtained by centrifuging the blood samples (without anticoagulant) at 1500×g for 15 min. The serum obtained was stored at − 20 °C for later use. The following biochemical parameters were evaluated: glucose, creatinine, urea, sodium, potassium, chloride, total pro- tein, albumin, globulin, bilirubin (bilirubin (T), bilirubin (D)), serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), triglyceride (TG), cholesterol (total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol). These parameters were evaluated using an automated biochemistry analyser and standard diagnostic test kits.

Statistical analysis

The results were calculated and expressed as Mean ± Standard deviation. The data obtained in the studies were subjected to one-way analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analysed using Dunnet's t-test. A p-value < 0.01 was considered to be significant.

Results

Preliminary phytochemical screening

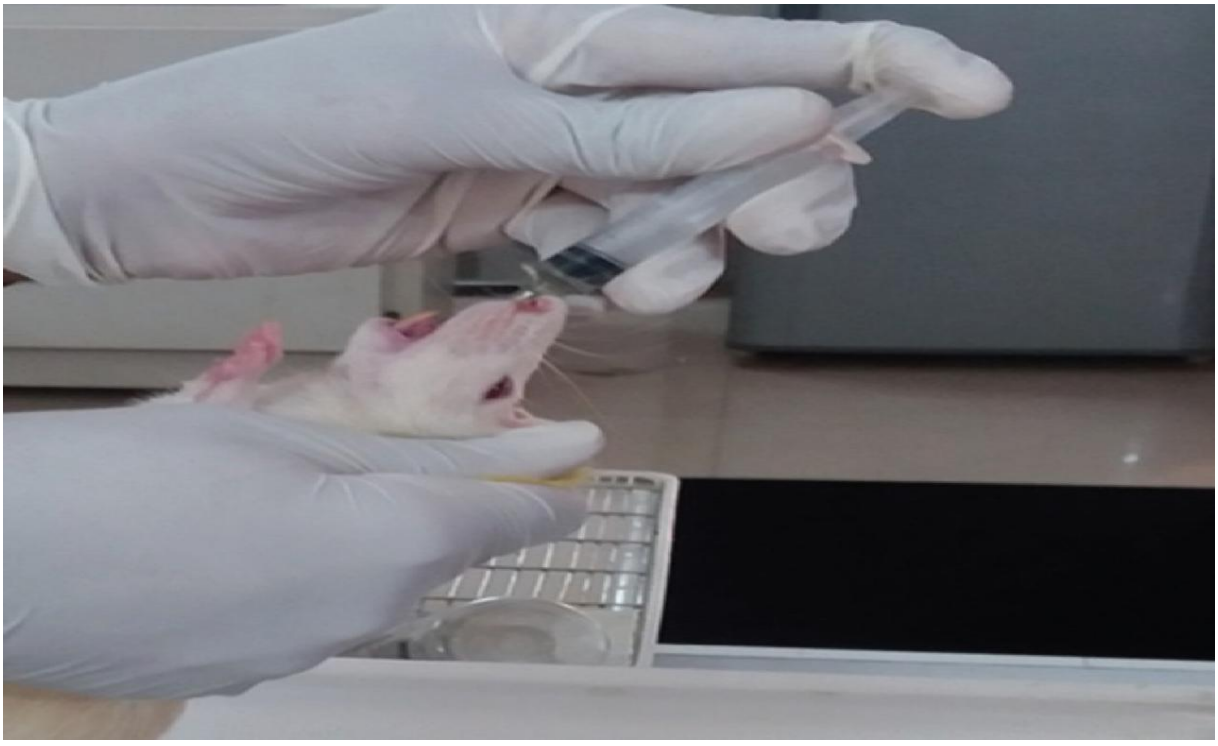
Preliminary phytochemical screening of CTAE and CTEE revealed the presence of major phytochemical groups such as alkaloids, carbohydrates, tannins, steroids and sterols, triterpenoids, saponins and flavonoids as shown in Table 4.1.1

Table no. 4.1.1: Preliminary phytochemical screening of three selected plant.

Chemical constituent	Chemical test	Bark of *Terminalia arjuna*		Flowers of *Chrysanthemum indicum*		Leaves of *Moringa oleifera*	
		Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
Proteins	Biuret test	-	+	-	+	+	+
Carbohydrate	Molish test	-	+	-	+	+	+
	Fehling's test	-	+	-	+	+	+
Alkaloid	Dragendorff's test	-	-	-	-	-	+
	Mayer's test	-	-	-	-	-	+
Steroids	Salkowaski test	+	+	-	-	-	-
	Liebermann-burchard test	+	+	-	-	-	+
Triterpene	Vanillin-sulphuric acid test	+	+	-	-	-	+
Tannin	Ferric chloride test	+	-	-	+	+	+
	Dilute nitric acid test	+	-	-	+	+	+
Glycoside	Keller-killani test	+	+	+	+	-	-
Flavonoid	Shinoda test	+	-	-	+	+	+
	Lead acetate test	+	-	-	+	+	+
Saponins	Foam formation test	-	-	-	-	+	+
Amino acids	Ninhydrin test	+	+	-	-	-	-

Acute toxicity study

In the acute toxicity studies, no mortality was observed within 4 h of continuous observation and also after 24 h. There was also no lethal effect observed after administration of both the CTAE and CTEE for the experimental period of 14 days. Morphological characteristics (fur, skin, eyes and nose) appeared normal. No salivation, diarrhoea, lethargy or unusual behaviours were observed. Food and water intake, body weight and respiration were normal. However, moderate sedation was observed after 10–15 min of administration of the CTAE dose of 2000 mg/kg per body weight (Limit dose). The sedative effect observed in the animals stayed for 30–40 minutes. There were no other toxic effects observed for both male and female rats within the study period.



This indicates that both the extract at the doses of 1000, 1500, and 2000 mg/kg, per body weight were safe. As there was no mortality recorded for all the doses, the LD50 value was assumed to be greater than the limit test dose of 2000 mg/kg, body weight. Hence 125, 250 and 500 mg/kg, oral doses of both CTAE as well as CTEE were selected to evaluate sub-acute toxicity study. The control group which was administered with normal vehicles (tween 20 solutions (1%, v/v)) did not produce any toxic effects or mortality within the study period.

Table No.4.2.1 Acute toxicity study for CTAE.

S.No.	Group	Dose in mg/kg	Mean body weight of animal (g)± SEM		
			Before dosing (1st Day)	After 1stweek (7th Day)	After 2nd week (14th Day)
1	Control	-	123.67±0.71	127.67±.49	131.33±0.61
2	CTAE	1000	125.28±2.12	127.16±1.18	130.14±0.21
3	CTAE	1500	124.13±1.18	126.18±0.14	131.13±0.32
4	CTAE	2000	126.17±1.11	132.33±1.12	139.33±0.76

Values are expressed as mean ± S.D. (n = 6). Statistical analysis done by one-way ANOVA followed by Dunnet's t-test.

Table No.4.2.2 Acute toxicity study for CTEE.

S.	Group	Dose in mg/kg	Mean body weight of animals (g)± SEM		
			Before dosing 1st Day)	After 1stweek (7th Day)	After 2nd week (14th Day)
1	Control	-	121.13±0.35	125.48±1.38	130.22±0.51
2	CTEE	1000	123.24±1.25	128.27±1.29	134.15±0.35
3	CTEE	1500	122.01±1.20	126.06±0.25	132.21±0.25
4	CTEE	2000	124.19±1.05	129.12±1.23	135.13±0.54

Values are expressed as mean ± S.D. (n = 6). Statistical analysis done by one-way ANOVA followed by Dunnet's t-test.

Sub-acute toxicity studies

As per the acute toxicity studies 125, 250 and 500 mg/kg, oral doses of both CTAE as well as CTEE were selected to evaluate sub-acute toxicity study.

Clinical observations and survival

No mortalities were recorded in rats over the period of 30 days of treatment with both the CTAE and CTEE at the doses of 125, 250 and 500 mg/kg, body weight, through oral route. None of the rats after administration of CTAE and CTEE at the doses of 125, 250 and 500 mg/kg body weight, showed any obvious morbidity or clinical symptoms of toxicity such as changes in the skin and fur, eyes, respiratory rate, autonomic (salivation, perspiration and piloerection), and stereotype activities throughout the experimental period of 30 days. There were no clinical signs of toxicity observed for the normal control group. Any minor changes or activities in animals found in the study period can be considered common findings for Wistar rats.

Body weight

The body weight of Wistar rats recorded at an interval of 5 days over the treatment period of 30 days and statistically significant increase in body weight was compared with the control is presented in Table 6.3.2.1 The results showed that in CTAE the body weight in the test extract treated groups increased non-significantly ($P > 0.05$) except in day 20 where the increase in body weight was significant ($P < 0.05$) for CTAE the dose of 500 mg/kg, b.wt. treated group when compared with the control.

In CTEE the increase in body weight was non-signifi- cant ($P > 0.05$) till day 15 except for the high dose treated group which showed significant ($P < 0.01$) in- crease on day 15. From day 20 to 30, the extract treated group showed significant increase in body weight when compared with the control. The increase in body weight for all groups was mostly dose dependant as a greater increase in body weight was observed in high dose for both the group.

Table No.4.3.2.1: Average Body weight for CTAE.

No.	Group	Dose in mg/kg	Mean body weight of animal (g)± SEM		
			1st Day	15th Day	30th Day
1	Control	-	123.67±0.71	128.67±.49	133.33±0.61
2	CTAE	125	125.28±2.12	130.16±1.18	135.14±0.21
3	CTAE	250	124.13±1.18	129.18±0.14	134.13±0.32
4	CTAE	500	126.17±1.11	131.33±1.12	136.33±0.76

Values are expressed as mean ± S.D. (n = 6). Statistical analysis done by one-way ANOVA followed by Dunnet's t-test.

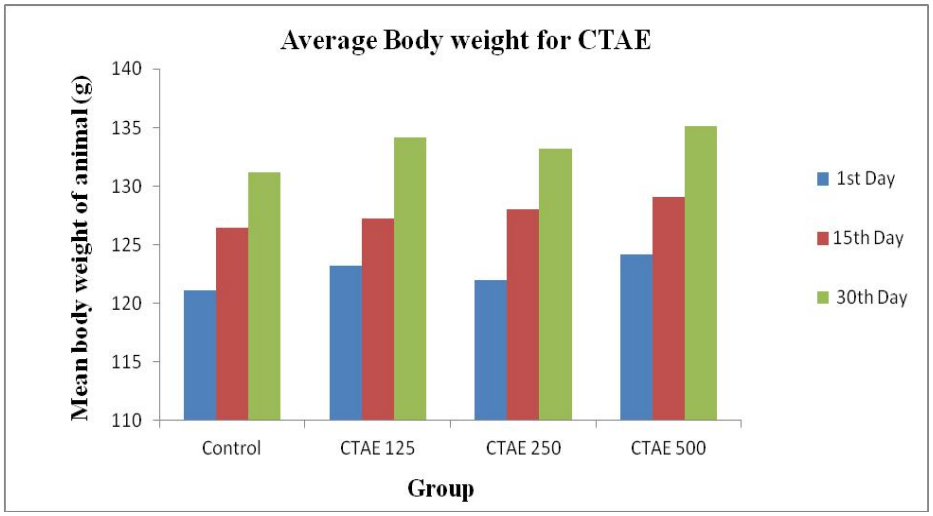


Figure No.4.3.2.1: Average Body weight for CTAE.

Table No.4.3.2 Average Body weight for CTEE.

No.	Group	Dose in mg/kg	Mean body weight of animals (g)± SEM		
			1st Day	15th Day	30th Day
1	Control	-	121.13±0.35	126.48±1.38	131.22±0.51
2	CTEE	125	123.24±1.25	127.27±1.29	134.15±0.35
3	CTEE	250	122.01±1.20	128.06±0.25	133.21±0.25
4	CTEE	500	124.19±1.05	129.12±1.23	135.13±0.54

Values are expressed as mean ± S.D. (n = 6). Statistical analysis done by one-way ANOVA followed by Dunnet's t-test.

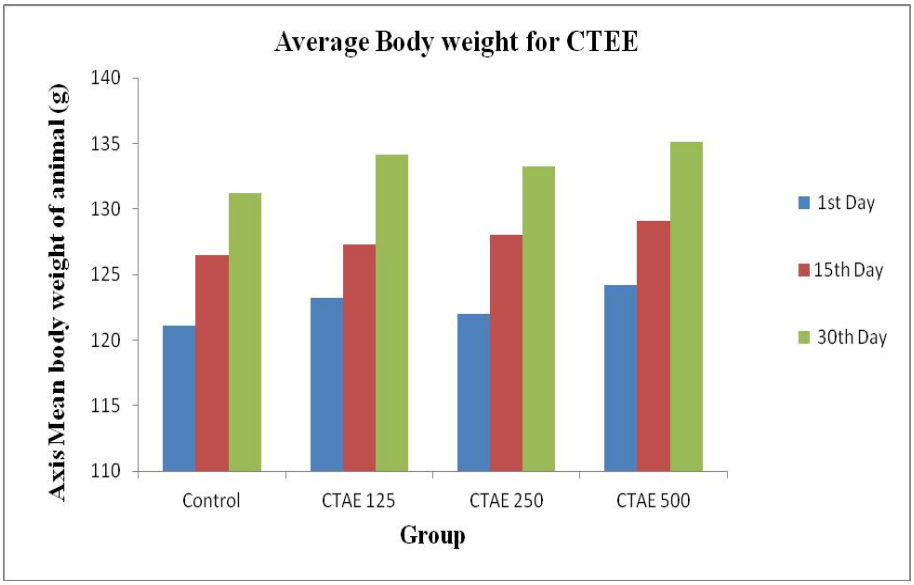


Figure No.4.3.2 Average Body weight for CTEE.

Haematological analysis

The effect of both the extract on haematological parameters of both male and female rats is tabulated in Table 2. Some significant changes were observed in the parameters of both male and female rats when compared with the control group. In male rats, increase in WBC count, RBC count, haematocrit (HCT), platelets (PLT), neutrophils and eosinophils were observed which are mainly non-significant and non-dose dependent. Decrease in haemoglobin and monocytes content were observed except for extract at a low dose which showed an in- crease in haemoglobin when compared with the control. Non-significant and non-dose dependant minor alter- ation in the values of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscu- lar haemoglobin concentration (MCHC), and lympho- cytes were observed in the male rats. In female rats, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), neutrophils and eosinophils increased mostly in a non-significant man- ner, whereas WBC content decreased non-significantly along with platelets (PLT) and lymphocytes count which showed significant ($P < 0.01$) decrease in a dose dependant manner when compared with the control. All the other parameters showed non-significant and non-dose dependent changes when compared with the control.

Table No. 4.3.3 Effects of CTAE and CTEE on biochemical parameters in rats treated for 30 days.

CTAE (Combination of Three Aqueous Extracts)					
Parameter	Unit	Control	125	250	500
Haemoglobin	gms/dL	13.64 ± 0.53	14.45 ± 0.69	12.58 ± 1.62	11.87 ± 1.04*
WBC	$10^3/\mu L$	8.89 ± 2.22	9.46 ± 0.95	11.86 ± 1.89*	11.42 ± 1.94
RBC	$10^6/\mu L$	8.22 ± 0.45	8.31 ± 0.61	8.33 ± 0.41	8.765 ± 0.21
Haematocrit (HCT)	%	38.33 ± 4.92	40.41 ± 4.17	45.83 ± 6.45*	46.17 ± 4.15*
Platelets (PLT)	$10^5/mm^3$	7.87 ± 0.66	7.83 ± 0.21	8.033 ± 0.36	8.27 ± 0.49
Mean corpuscular volume (MCV)	fL	52.46 ± 2.71	50.38 ± 2.80	50.28 ± 3.49	52.99 ± 2.53
Mean corpuscular haemoglobin (MCH)	pg	17.73 ± 0.91	17.95 ± 0.88	17.36 ± 0.46	17.93 ± 0.74
Mean corpuscular hemoglobin concentration (MCHC)	%	29.11 ± 1.81	29.01 ± 2.48	29.2 ± 2.87	26.4 ± 2.51
Differential leucocyte count					
Neutrophils	%	20.5 ± 6.92	18.67 ± 5.82	24.33 ± 5.82	23.83 ± 4.02
Lymphocytes	%	73.67 ± 7.09	72.5 ± 6.09	74 ± 6.13	73.16 ± 3.97
Eosinophils	%	1.5 ± 0.83	2.33 ± 1.03	4.67 ± 1.51**	4.33 ± 0.81**
Monocytes	%	2.83 ± 1.33	2.5 ± 1.05	2.17 ± 1.17	2.66 ± 1.75
CTAE (Combination of Three Ethanolic Extracts)					
Haemoglobin	gms/dL	13.66 ± 0.86	13.41 ± 0.61	13.80 ± 0.71	14.13 ± 0.68
WBC	$10^3/\mu L$	8.19 ± 1.78	8.25 ± 1.13	7.90 ± 1.35	7.17 ± 1.18
RBC	$10^6/\mu L$	7.34 ± 0.70	7.39 ± 0.56	7.30 ± 0.51	7.99 ± 0.44
Haematocrit (HCT)	%	41.51 ± 2.74	41.19 ± 2.72	39.96 ± 2.92	43.49 ± 1.76
Platelets (PLT)	$10^5/mm^3$	7.54 ± 0.45	6.065 ± 0.52**	4.34 ± 0.47**	3.055 ± 0.35**
Mean corpuscular volume (MCV)	fL	53.18 ± 3.71	53.39 ± 3.57	54.16 ± 4.11	58.64 ± 2.79*
Mean corpuscular haemoglobin (MCH)	pg	18.08 ± 1.40	19.36 ± 1.17	18.51 ± 1.88	20.61 ± 1.31*
Mean corpuscular hemoglobin concentration (MCHC)	%	33.72 ± 2.11	32.21 ± 2.31	32.18 ± 1.70	34.15 ± 1.58
Differential leucocyte count					
Neutrophils	%	20.93 ± 4.33	32.51 ± 3.04**	37.93 ± 5.43**	51.21 ± 4.47**
Lymphocytes	%	71.82 ± 5.76	53.75 ± 5.62**	49.5 ± 4.31**	41.46 ± 4.79**
Eosinophils	%	1.64 ± 0.77	3.13 ± 1.15	2.65 ± 1.36	4.5 ± 0.77**
Monocytes	%	2.61 ± 0.55	2.50 ± 0.66	2.76 ± 0.30	2.57 ± 0.48

Biochemical analysis

Table portrays the biochemical parameters of both male and female rats. The result in male rats showed a dose dependent increase in glucose and creatinine level which was significant in high dose. Triglycerides and cholesterol levels (Total cholesterol, HDL cholesterol, LDL cholesterol, and VLDL cholesterol) increased mostly significantly in a non-dose dependent manner when compared with the control. Urea and total protein decreased significantly in all doses whereas ALP and SGPT levels decreased significantly only in the high dose group. All the other biochemical parameters showed minor changes which are mostly non-significant and non-dose dependent. In female rats, a decrease in glucose, urea, albumin, SGPT, SGOT, and ALP was observed which were mainly significant when com- pared to control. Creatinine, bilirubin (both total and direct), triglyceride, and cholesterol levels (Total chol- esterol, HDL cholesterol, LDL cholesterol, and VLDL cholesterol) increased mostly non-significantly in a non-dose dependant manner when compared with the control. The rest of the parameters showed minor fluctua- tions mostly non-significantly when compared with the control.

Table 4.3.4.1 Effects of Extract on biochemical parameters in rats treated for 30 days.

Parameter	Unit	Control	Dose mg/kg		
			125	250	500
CTAE (Combination of Three Aqueous Extracts)					
Glucose	mg/dL	73.82 ± 5.11	75.18 ± 4.22	75.59 ± 4.24	80.99 ± 1.46*
Creatinine	mg/dL	0.48 ± 0.14	0.65 ± 0.11	0.70 ± 0.20	0.88 ± 0.14**
Urea	mg/dL	34.78 ± 2.88	29.83 ± 2.64*	28.68 ± 2.51**	28.09 ± 2.94**
Sodium	mmol/L	146.97 ± 14.61	145.73 ± 12.90	144.10 ± 8.38	147.23 ± 5.11
Potassium	mmol/L	6.27 ± 1.85	6.56 ± 1.17	6.10 ± 1.18	5.64 ± 0.76
Chloride	mmol/L	110.82 ± 8.53	106.83 ± 5.14	111.04 ± 6.11	109.69 ± 9.50
Total protein	g/dL	6.52 ± 0.41	6.01 ± 0.20**	5.90 ± 0.16**	5.92 ± 0.16**
Albumin	g/dL	3.51 ± 0.31	3.29 ± 0.35	3.54 ± 0.29	3.76 ± 0.21
Globulin	mg/dL	2.86 ± 0.20	2.64 ± 0.42	2.87 ± 0.58	2.43 ± 0.32
Bilirubin					
Bilirubin (T)	mg/dL	0.76 ± 0.20	0.79 ± 0.23	0.79 ± 0.15	0.75 ± 0.12
Bilirubin (D)	mg/dL	0.08 ± 0.02	0.103 ± 0.02	0.106 ± 0.02	0.111 ± 0.04
SGPT	IU/L	33.10 ± 5.62	32.04 ± 5.21	29.06 ± 2.96	26.94 ± 4.00
SGOT	IU/L	63.95 ± 7.76	65.11 ± 4.39	59.79 ± 5.99	48.51 ± 3,48**
ALP	IU/L	145.94 ± 11.13	143.51 ± 7.16	136.51 ± 11.82	122.36 ± 6.88**
Triglyceride (TG)	mg/dL	80.40 ± 10.17	94.34 ± 6.97*	99.20 ± 6.94**	98.001 ± 5.20**
Cholesterol					
Total cholesterol	mg/dL	64.5 ± 5.90	108.64 ± 8.44**	128.3 ± 6.74**	135.33 ± 6.58**
HDL cholesterol	mg/dL	31.40 ± 2.39	35.86 ± 3.91*	37.49 ± 3.18**	38.77 ± 2.06**
LDL cholesterol	mg/dL	31.59 ± 2.86	30.27 ± 1.66	31.18 ± 2.05	32.69 ± 2.44
VLDL cholesterol	mg/dL	15.45 ± 1.49	15.75 ± 2.13	18.12 ± 1.19*	20.99 ± 1.38**
CTAE (Combination of Three Ethanolic Extracts)					
Glucose	mg/dL	86.59 ± 7.01	80.88 ± 3.81	76.23 ± 5.81*	72.68 ± 7.03**
Creatinine	mg/dL	0.61 ± 0.19	0.78 ± 0.12	0.7 ± 0.12	0.86 ± 0.06**
Urea	mg/dL	41.40 ± 7.75	28.75 ± 3.00**	30.45 ± 2.21**	26.99 ± 3.58**
Sodium	mmol/L	142.006 ± 4.80	140.15 ± 1.51	140.05 ± 3.81	141.07 ± 2.56
Potassium	mmol/L	6.39 ± 1.49	6.46 ± 0.51	6.27 ± 0.41	6.07 ± 0.23
Chloride	mmol/L	104.57 ± 6.76	107.09 ± 4.80	105.96 ± 5.62	106.03 ± 5.83
Total protein	g/dL	6.30 ± 0.45	6.11 ± 0.41	6.13 ± 0.39	5.96 ± 0.21
Albumin	g/dL	3.93 ± 0.39	3.81 ± 0.19	3.77 ± 0.29	3.73 ± 0.16
Globulin	mg/dL	3.05 ± 0.39	2.97 ± 0.21	3.17 ± 0.21	2.50 ± 0.35*
Bilirubin					
Bilirubin (T)	mg/dL	0.58 ± 0.13	0.67 ± 0.12	0.69 ± 0.15	0.84 ± 0.08**
Bilirubin (D)	mg/dL	0.14 ± 0.04	0.15 ± 0.04	0.17 ± 0.04	0.16 ± 0.04
SGPT	IU/L	51.60 ± 8.66	40.92 ± 5.63*	40.80 ± 5.88*	37.16 ± 6.29**
SGOT	IU/L	43.92 ± 5.92	42.29 ± 3.61	38.22 ± 2.76	33.39 ± 3.57**
ALP	IU/L	129.07 ± 10.45	125.25 ± 5.09	120.77 ± 4.41	119.81 ± 6.26
Triglyceride (TG)	mg/dL	73.27 ± 8.03	94.09 ± 9.05**	104.02 ± 9.35**	112.60 ± 9.99**
Cholesterol					
Total cholesterol	mg/dL	91.12 ± 8.67	105.89 ± 5.06**	115.15 ± 6.46**	114.02 ± 5.75**
HDL cholesterol	mg/dL	35.65 ± 4.54	36.33 ± 2.88	36.25 ± 2.87	40.72 ± 1.58*
LDL cholesterol	mg/dL	41.42 ± 4.46	43.53 ± 3.76	44.47 ± 1.84	46.11 ± 5.16
VLDL cholesterol	mg/dL	17.11 ± 2.54	18.03 ± 2.07	20.94 ± 3.04*	21.63 ± 2.40*

CONCLUSION

The present investigation of the combination of three aqueous (CTAE) & ethanolic extracts (CTEE) which include Bark of *Terminalia arjuna*, flowers of *Chrysanthemum indicum* and leaves of *Moringa oleifera was* selected as a sample material. The phytochemical investigation revealed the presence of bioactive compounds such as phenolic, flavonoids, terpenoids, steroids, alkaloids and glycoside in the CTAE & CTEE. The hyperlipidemic action of the CTEE was found to be more potent than the CTAE. In toxicity studies with no mortality recorded for all the doses, the LD50 value was assumed to be greater than the limit test dose of 2000 mg/kg, body weight. Hence 125, 250 and 500 mg/kg, oral doses of both CTAE as well as CTEE were selected for study of antihyperlipidemic activity.

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ABBREVIATION

CTAE : Combination of Three Aqueous Extracts (bark of *Terminalia arjuna*, flowers of *Chrysanthemum indicum* and leaves of *Moringa oleifera)*
CTEE : Combination of Three Ethanolic Extracts (bark of *Terminalia arjuna*, flowers of *Chrysanthemum indicum* and leaves of *Moringa oleifera)*
EDTA : Ethylenediaminetetraacetic acid
OECD : Organization for Economic Co-operation and Development
CPCSEA:Committee for the Purpose of Control and Supervision of Experiments on Animals
VLDL : Very low density lipoproteins
HDL : High density lipoproteins
LDL : Low density lipoproteins

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