Acute oral toxicity evaluation of Hydro alcoholic extract of Mesua ferrea L. leaves in albino mice as per OECD 423

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Abstract:

Traditionally mesua ferrea Linn has been to treat various diseases .Many of its medicinal uses have been reported scientifically, But its toxicity have never been reported.The design of the study is to find out the severe toxic potential of the hydroalcoholic extract of mesua ferrea linn as per OECD 423.Female mice are divided into four groups (n=5).Group I serve as control which receive normal saline while group II, III.IV are treated with hydroalcoholic extract of the leaves of mesua ferrea linn in a dose of 500 mg/kg, 1000 mg/kg, 2000 mg /kg body weight respectively .All the groups observed for the period of 14 days. The ill health /over toxicity will be monitored once duing the first 30 Minuits of dosing, periodically for first 24 hours and thereafter daily for 14 days. On day 15th, all animals were anesthetized and blood samples were collected for estimation of hematological and clinical chemistry parameters. Following blood collection the animals were humanly sacrificed using Ketamine overdose, subjected to the gross necropsy and specified organs were collected and preserved in 10% NBF for histopathological examination. Since there was no observed toxic effect of test sample at the highest dose of 2000 mg /kg because all the animals are safe and there is no periodical difference was observed in the body weight of animals. There was no significant difference was noticed in haematological and clinical parameters. From the collected data it is interpreted that the LD50 is greater than 2000 mg/kg body weight. Even the moderate toxicity is not appeared which is concluded from all the profile.

Keywords: Mesua ferrea L, LD $_{50}$, Acute oral toxicity,oecd423

1. Introduction

Human beings returned to traditional medicines in recent years particularly for treating and preventing the diseases effectively by using natural products without any side effects .The bacterial resistance of the many antibiotics is highly developed is in quandary which will be the reason to turn towards naturally derived products from medicinal plants with more productive when compared to synthetic drugs [1]

From many centuries of drug development the role of medicinal plant on pharmacological research is highly increased. The bioactive compound present in the plant are sophisticated

under the pathways of secondary metabolism .They used as prophylactic agents and therapeutics and also used as starting material for synthesis of drugs or as a replica for the compounds which has highly pharmacological action. [2]

The genus mesua (callophyllacea)is widely used for many ailments such as antipyretic ,antiasthmatic ,antiallergic ,cardiotonic ,anti-inflammatory,hepotoprotective ,antispasmodic ,and immunosuppressant activity .The presence of triterpenoids ,flavonoids ,fats ,coumarin phenyl coumarin and xanthones responsible for its biological activity . **[**3**]**

Many pharmacologically active medicinal plant are utilised for human health .The toxic effect may produce when consumed without proofed scientifically ,so that the potential toxicity screening of medicinal products obtained from naturals is seeking more attention [4].Over the long tenure use of natural products with lack of health risk which consider that the medicine is harmless [5,6]. Rather than the use of traditional medicine used widely ,there is no research has developed which may explain the toxic effects of all plants .The toxicity study of mesua ferrea linn is systematically lacking even it has been used in medicine from many years particularly in alternative system of medicine was the intent of the research work .

2 .Materials and methods

2.1 Plant collection and extraction

The plant was collected from the foot hills of Western Ghats of Palakkad district from Kerala. The plant specimen was identified and authenticated by the government arts and science college ooty, the nilgiris Tamilnadu. The leaves were washed, shade dried, powdered and stored in airtight container for future use. 50 g of powdered leaves were extracted with aqueous alcohol in a soxhelet extractor by continuous hot percolation method. Extracts were concentrated by rotary vacuum evaporator and the residue obtained was dried, weighed

2.2 Animals and ethical committee approval

Adult Swiss albino mice (8 to 9-week-old female) weighing 20–25 g were obtained from the Central Animal House Facility, Skanda life sciences, Bengaluru, India. The animal experiments were approved by the Institutional Animal Ethical Committee, Skanda life sciences (Organization), Bengaluru, India (Approval number: **IAEC-SLS-2021-038,**), for the purpose of acute oral toxicity study. During all experiments, animal care and handling were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

2.3 Acute oral toxicity assay

After acclimatization period, the animals were equally distributed into 4 groups of 5 animals per group. Animals were fasted overnight prior to dosing (feed but not water was withheld). The test item was administered orally to GII, GIII and GIV group animals on Day 1 of study at 500 mg/kg B.wt, 1000 mg/kg B.wt, and 2000 mg/kg B.wt, respectively. GI group animals were treated as control and were administered with vehicle alone orally. Animals were observed individually after the dosing during first 30 minutes, periodically during the first 24 hours and daily thereafter, for a total of 14 days. The body weight of the animal, the food and water intake of the animals was closely monitored and it is recorded. On day 15th, all animals were anesthetized and blood samples were collected for estimation of haematological and clinical chemistry parameters. Following blood collection the animals were humanly sacrificed using Ketamine overdose, subjected to the gross necropsy and specified organs were collected and preserved in 10% NBF for histopathological examination**.**

2.4 Body weight of the animals

The body weight of the animals are recorded individually in day 1.day 7, day 14 of the day**.**

2.5 Feed and water Consumption

Animal were group housed in cages as per dose and were provided with *ad libitum* feed and water. Feed and water consumption was recorded weekly in animals group wise.

2.6 Biochemical analysis

Total Bilirubin (TB),Direct Bilirubin(DB),Indirect Bilirubin(IB), Total Protein(TP), Serum Albumin(SA), Serum Globulin (SG),Albumin-Globulin Ratio(A/G), Aspartate amino transferase(AST), Alanine amino transferase(ALT),Alkaline Phosphatase(ALP), Serum Creatinine (SC), Blood Urea Nitrogen (BUN) were measured .

2.7 Haematological analysis

From all the animals (both treated and control groups)the blood samples were collected in EDTA containing tubes for haematological study, The CBC parameters like Haemoglobin (Hb),White Blood Cells, Neutrophils(WBC),Lymphocytes (L)Eosinophil's(E),Platelet counts(PC) , Red Blood Cells (RBC), Packed Cell Volume(PCV), Mean Corpuscular Volume(MCV),Mean Corpuscular Haemoglobin(MCH) Mean Corpuscular Haemoglobin Concentration(MCHC) were determined with humalyzer .

2.8 Statistical analysis

Experimental results were presented as mean ± SEM and the statistical significance between the groups was analysed by means of one way ANOVA followed by Turkey's multiple comparison test. $P \leq 0.05$was considered as statistically significant

3 Results

3.1 Clinical observation:

Since there was no observed toxic effect of test sample at the highest dose of 2000 mg /kg because all the animals are safe and there is no periodical difference were observed in the body weight of animals .The feed and water consumption of the animals are gradually increased when it is tested in the day 1, 7, and day 14th .There was no significant difference were observed in hematological and biochemical parameters.

Table 1: Summary of clinical signs and mortality in animals during 14 days observation period

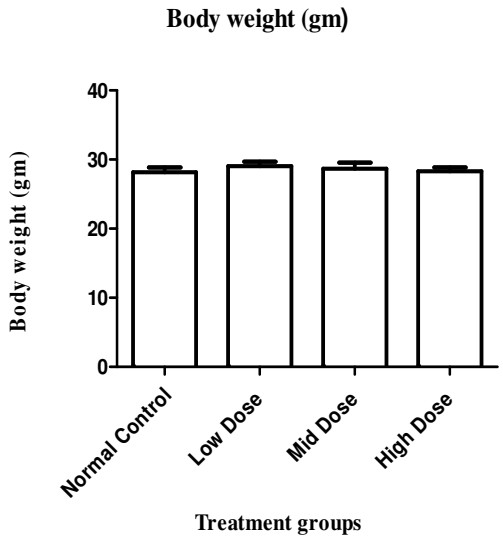
		Study Day		
Group	**Treatment and Dose**	**1 to 7**	**8 to 14**	**Mortality**
G-I	Normal Control	NAD	NAD	0/5
G-II	Low Dose (500 mg/kg B.wt)	NAD	NAD	0/5
G-III	Mid Dose (1000 mg/kg B.wt)	NAD	NAD	0/5
G-IV	High Dose (2000 mg/kg B.wt)	NAD	NAD	0/5

NAD – No Abnormality Detected

Table – 2: Summary of body weight of animals in Mean ± SD (gm)

		Day		
Group	**Treatment and Dose**	**1**	**7**	**14**
G-I	Normal Control	23.18 ± 0.85	26.78 ± 1.69	28.14 ± 1.52
G-II	Low Dose (500 mg/kg B.wt)	23.28 ± 0.85	25.34 ± 1.25	29.02 ± 1.52
G-III	Mid Dose (1000 mg/kg B.wt)	23.3 ± 0.64	25.2 ± 1.44	28.64 ± 1.94
G-IV	High Dose (2000 mg/kg B.wt)	23.2 ± 0.89	25.56 ± 1.99	28.3 ± 1.15

n=5; Values are Mean ± Standard Deviation



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	-0.8800	0.8938	No	ns	-3.432 to 1.672
Normal Control vs Mid Dose	-0.5000	0.5079	No	ns	-3.052 to 2.052
Normal Control vs High Dose	-0.1600	0.1625	No	ns	-2.712 to 2.392

Fig 1: One way ANOVA analysis of Body weight between the treatment groups

Table-3: Summary of group wise weekly feed consumption in animals

		Weekly feed consumption (gm/group)	
Group	**Treatment and Dose**	**Day 1 to 7**	**Day 8 to 14**
G-I	Normal Control	158	166.1
G-II	Low Dose (500 mg/kg B.wt)	156.1	163.4
G-III	Mid Dose (1000 mg/kg B.wt)	154	161.1
G-IV	High Dose (2000 mg/kg B.wt)	150.6	158.9

n=5; Values are weekly feed consumption per group in gram.

Table – 4: Summary of weekly water consumption in animals

		Weekly water consumption (ml/group)	
Group	**Treatment and Dose**	**Day 1 to 7**	**Day 8 to 14**
G-I	Normal Control	128.7	130.1
G-II	Low Dose (500 mg/kg B.wt)	126.3	127.6
G-III	Mid Dose (1000 mg/kg B.wt)	126.6	129.1
G-IV	High Dose (2000 mg/kg B.wt)	128.3	130

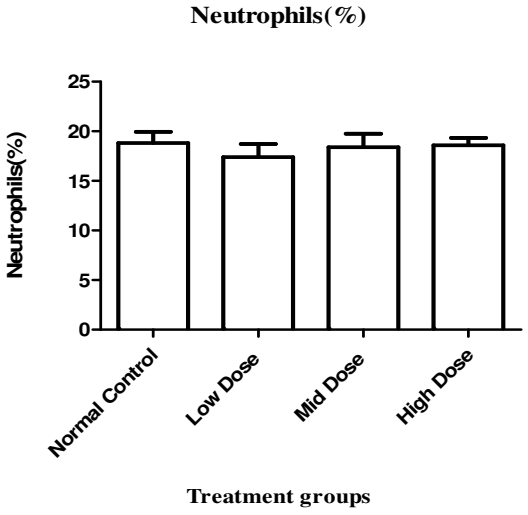
n=5; Values are weekly water consumption per group in ml.

Table – 5: Summary of Haematological Parameters

Group	**Treatment and Dose**	**Hb (gm %)**	**WBC (cells/ cumm)**	**Neutrophils (%)**	**Lymphocytes (%)**	**Eosinophils (%)**	**Platelet count (Lakhs/cumm)**	**RBC Count (mill/cumm)**	**PCV (%)**	**M.C.V (fl)**	**M.C.H (pg)**	**M.C.H.C (%)**
G-I	**NC**	14.92 ± 0.46	11.68 ± 0.47	18.8 ± 1.16	58.6 ± 3.06	2 ± 0.32	8.6 ± 0.76	8.96 ± 0.23	42.04 ± 0.77	50.92 ± 2.58	15.26 ± 0.17	25.44 ± 0.67

G-II	**LD**	14.68 ± 0.37	11.64 ± 1.08	17.4 ± 1.33	57.2 ± 1.53	1.8 ± 0.37	9.18 ± 0.32	9.36 ± 0.48	42.16 ± 1.12	52.72 ± 3.25	15.94 ± 0.41	26.08 ± 0.31
G-III	**MD**	14.56 ± 0.34	11.6 ± 0.57	18.4 ± 1.36	58.4 ± 1.44	2 ± 0.32	8.45 ± 0.69	9.14 ± 0.39	41.66 ± 0.69	53.02 ± 1.13	16.16 ± 0.44	26.32 ± 0.36
G-IV	**HD**	15.06 ± 0.53	11.58 ± 0.83	18.6 ± 0.75	59 ± 1.3	2.2 ± 0.37	9.14 ± 0.74	8.92 ± 0.4	43.22 ± 1.05	52.66 ± 2.59	15.46 ± 0.24	25.96 ± 0.48

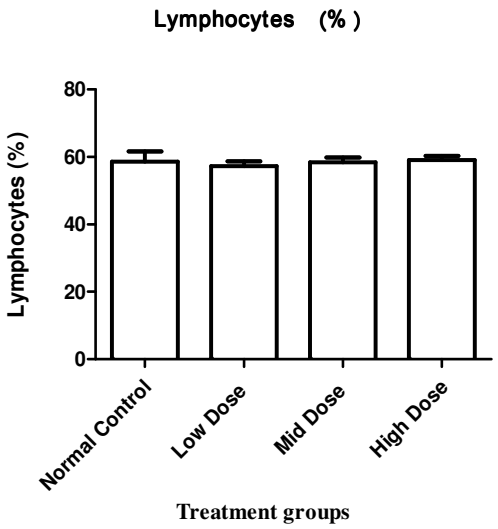
n=5; Values are Mean ± Standard Error of mean



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	1.400	0.8427	No	ns	-2.907 to 5.707
Normal Control vs Mid Dose	0.4000	0.2408	No	ns	-3.907 to 4.707
Normal Control vs High Dose e	0.2000	0.1204	No	ns	-4.107 to 4.507

Fig 2: One way ANOVA analysis of Neutrophils between the treatment groups

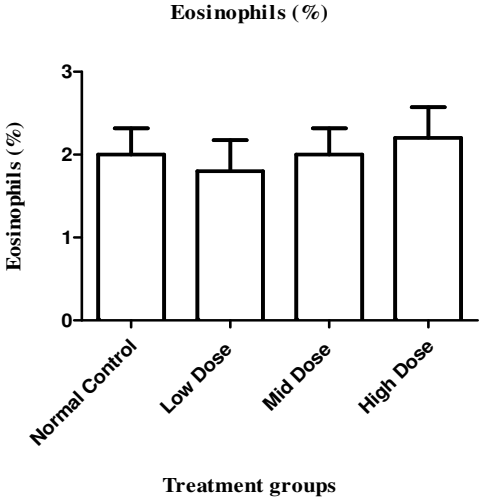
No significant difference was observed in Neutrophils values of treatment group as compared to normal control group.



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	1.400	0.5035	No	ns	-5.808 to 8.608
Normal Control vs Mid Dose	0.2000	0.07193	No	ns	-7.008 to 7.408
Normal Control vs High Dose e	-0.4000	0.1439	No	ns	-7.608 to 6.808

Fig 3: One way ANOVA analysis of Lymphocytes between the treatment groups

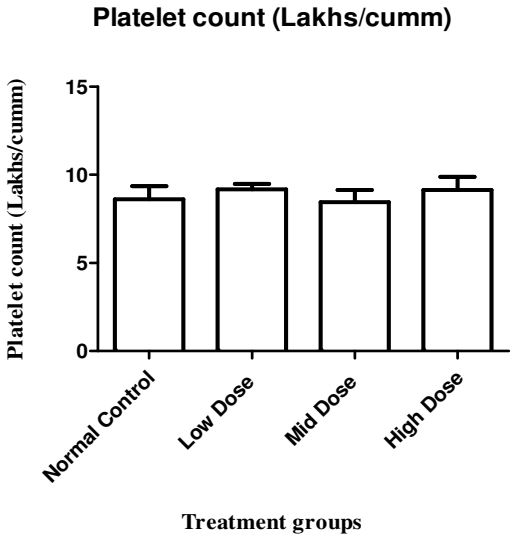
No significant change was observed in Lymphocytes values of treatment groups as compared to normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	0.2000	0.4082	No	ns	-1.070 to 1.470
Normal Control vs Mid Dose	0.0000	0.0000	No	ns	-1.270 to 1.270
Normal Control vs High Dose e	-0.2000	0.4082	No	ns	-1.470 to 1.070

Fig 4: One way ANOVA analysis of Eosinophils between the treatment groups

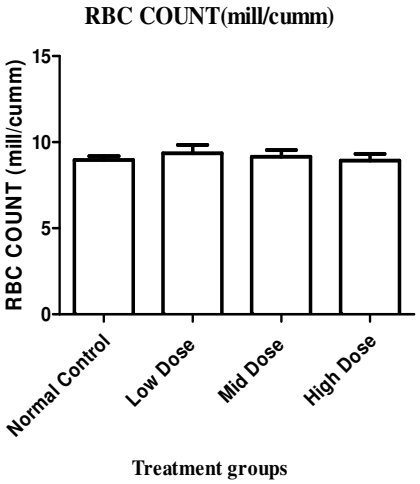
No significant difference was observed in Eosinophils values of treatment groups when compared with normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	-0.5760	0.6250	No	ns	-2.965 to 1.813
Normal Control vs Mid Dose	0.1580	0.1715	No	ns	-2.231 to 2.547
Normal Control vs High Dose e	-0.5380	0.5838	No	ns	-2.927 to 1.851

Fig 5: One way ANOVA analysis of Platelet count between the treatment groups

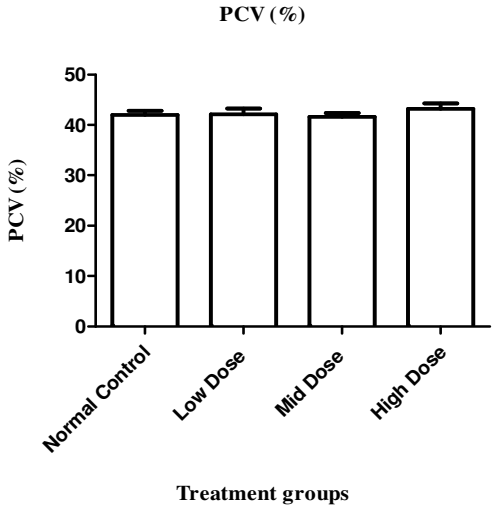
No significant difference was observed in Platelet count values of treatment groups as compared to normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	-0.4000	0.7302	No	ns	-1.820 to 1.020
Normal Control vs Mid Dose	-0.1800	0.3286	No	ns	-1.600 to 1.240
Normal Control vs High Dose e	0.04000	0.07302	No	ns	-1.380 to 1.460

Fig 6: One way ANOVA analysis of RBC Count between the treatment groups

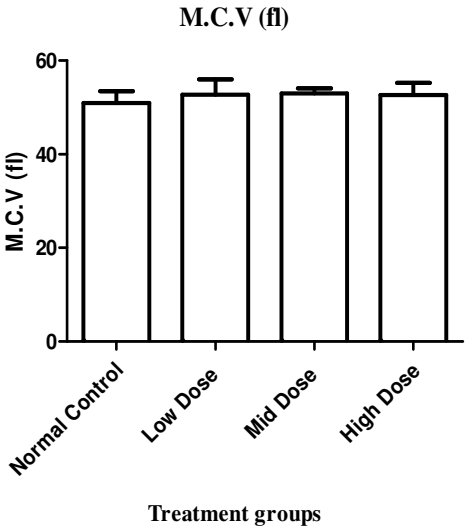
No significant change was observed in RBC Count values of treatment groups as compared to normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	-0.1200	0.09167	No	ns	-3.514 to 3.274
Normal Control vs Mid Dose	0.3800	0.2903	No	ns	-3.014 to 3.774
Normal Control vs High Dose e	-1.180	0.9014	No	ns	-4.574 to 2.214

Fig 7: One way ANOVA analysis of PCV between the treatment groups

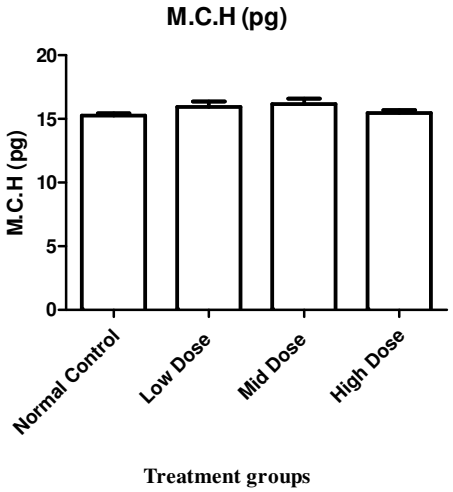
No significant difference was observed in PCV values of treatment groups as compared to normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	-1.800	0.5073	No	ns	-11.00 to 7.398
Normal Control vs Mid Dose	-2.100	0.5919	No	ns	-11.30 to 7.098
Normal Control vs High Dose e	-1.740	0.4904	No	ns	-10.94 to 7.458

Fig 8: One way ANOVA analysis of M.C.V between the treatment groups

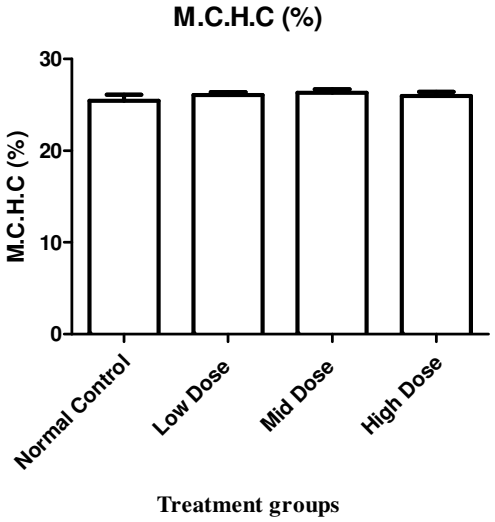
No significant difference was observed in M.C.V values of treatment groups as compared to normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	-0.6800	1.439	No	ns	-1.905 to 0.5448
Normal Control vs Mid Dose	-0.9000	1.905	No	ns	-2.125 to 0.3248
Normal Control vs High Dose e	-0.2000	0.4233	No	ns	-1.425 to 1.025

Fig 9: One way ANOVA analysis of M.C.H between the treatment groups

No significant difference was observed in M.C.H values of treatment groups as compared to normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	-0.6400	0.9530	No	ns	-2.381 to 1.101
Normal Control vs Mid Dose	-0.8800	1.310	No	ns	-2.621 to 0.8610
Normal Control vs High Dose e	-0.5200	0.7743	No	ns	-2.261 to 1.221

Fig 10: One way ANOVA analysis of M.C.H.C between the treatment group

No significant change was observed in M.C.H.C values of treatment groups as compared to normal control group

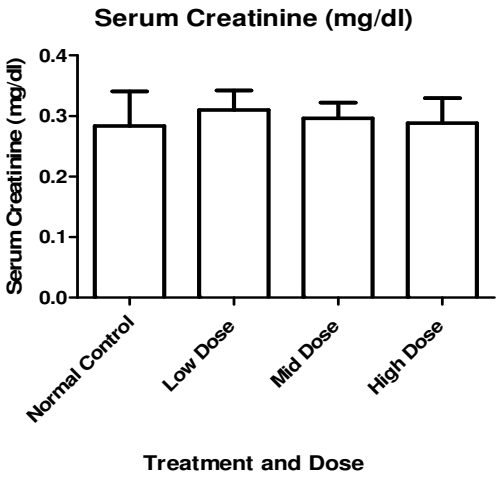
Table – 6: Summary of Clinical parameters

Group	Treatment and Dose	TB (mg/dl)	TP (g/dl)	SA (g/dl)	SG (g/dl)	A/G	AST (U/L)	ALT (U/L)	ALP (IU/L)	SC (mg/dl)	BUN (mg/dl)
G-I	**NC**	0.33 ± 0.06	6.07 ± 0.15	3.47 ± 0.09	2.6 ± 0.1	1.34 ± 0.05	111.8 ± 5.81	58.5 ± 5.59	147.4 ± 11.4	0.28 ± 0.05	21.06 ± 0.67
G-II	**LD**	0.35 ± 0.06	6.17 ± 0.26	3.31 ± 0.08	2.86 ± 0.22	1.19 ± 0.09	107.4 ± 4.2	56.5 ± 3.38	135.4 ± 3.01	0.31 ± 0.03	19.94 ± 0.44
G-III	**MD**	0.38 ± 0.04	6.04 ± 0.14	3.38 ± 0.07	2.66 ± 0.12	1.28 ± 0.07	108.2 ± 4.54	58.06 ± 3.3	146.4 ± 10.01	0.3 ± 0.02	20.96 ± 0.98
G-IV	**HD**	0.38 ± 0.06	5.74 ± 0.16	3.21 ± 0.03	2.53 ± 0.15	1.29 ± 0.08	106.8 ± 3.87	57.8 ± 3.46	142.2 ± 9.49	0.29 ± 0.04	20.9 ± 1.12

n=5; Values are Mean ± Standard Error of mean

NC- Normal Control, LD-Low Dose (500 mg/kg B.wt), MD- Mid Dose (1000 mg/kg B.wt), HD-High Dose (2000 mg/kg B.wt)

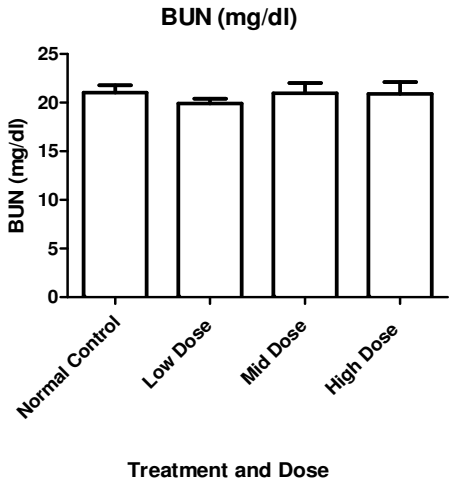
TB – Total Bilirubin, DB – Direct Bilirubin, IB – Indirect Bilirubin, TP – Total Protein, SA – Serum Albumin, SG – Serum Globulin, A/G – Albumin-Globulin Ratio, AST – Aspartate amino transferase, ALT – Alanine amino transferase, ALP – Alakaline Phosphatase, SC – Serum Creatinine, BUN – Blood Urea Nitrogen.



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	-0.02600	0.4510	No	ns	-0.1754 to 0.1234
Normal Control vs Mid Dose	-0.01200	0.2082	No	ns	-0.1614 to 0.1374
Normal Control vs High Dose	-0.004000	0.06939	No	ns	-0.1534 to 0.1454

Fig 11: One way ANOVA analysis of Serum Creatinine between the treatment group

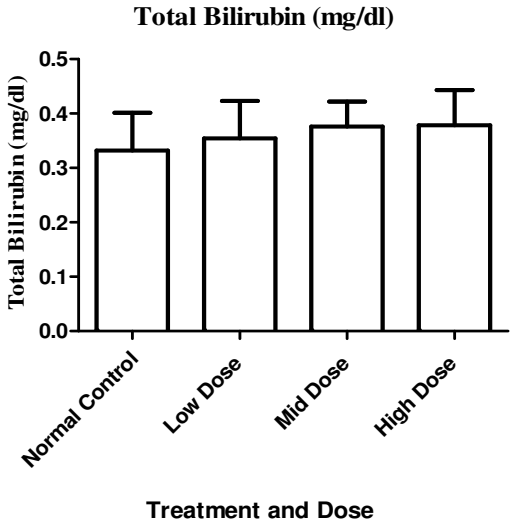
No significant change was noticed in Serum Creatinine values of treatment groups as compared with normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	1.124	0.8589	No	ns	-2.269 to 4.517
Normal Control vs Mid Dose	0.1000	0.07642	No	ns	-3.293 to 3.493
Normal Control vs High Dose	0.1600	0.1223	No	ns	-3.233 to 3.553

Fig 12: One way ANOVA analysis of BUN between the treatment group

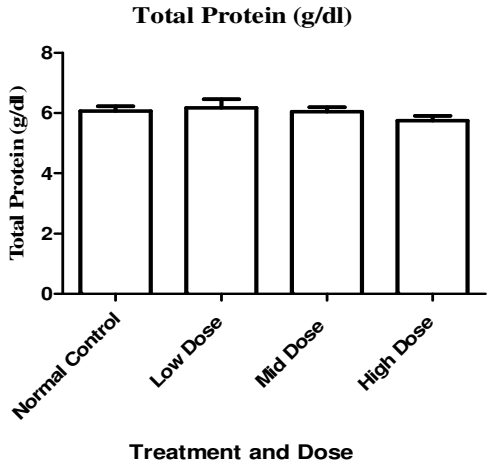
No significant change was noticed in BUN values of treatment groups when compared with normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	-0.02200	0.2462	No	ns	-0.2537 to 0.2097
Normal Control vs Mid Dose	-0.0440	0.4924	No	ns	-0.2757 to 0.1877
Normal Control vs High Dose	-0.0460	0.5148	No	ns	-0.2777 to 0.1857

Fig 13: One way ANOVA analysis of Total Bilirubin between the treatment group

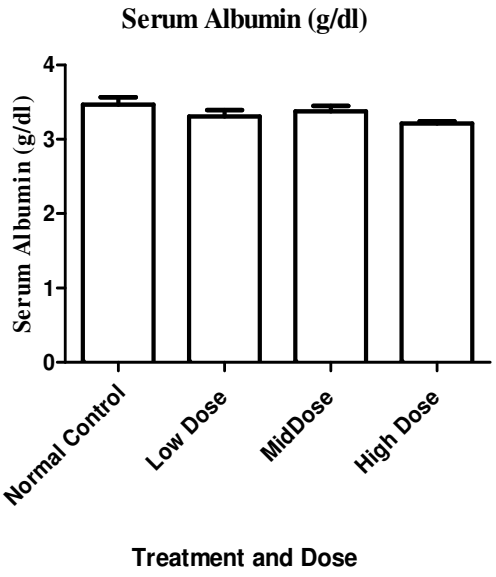
No significant change was noticed in Total Bilirubin values of treatment group when compared with normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	-0.1060	0.3738	No	ns	-0.8411 to 0.6291
Normal Control vs Mid Dose	0.02400	0.08464	No	ns	-0.7111 to 0.7591
Normal Control vs High Dose	0.3280	1.157	No	ns	-0.4071 to 1.063

Fig 14: One way ANOVA analysis of Total Protein between the treatment groups

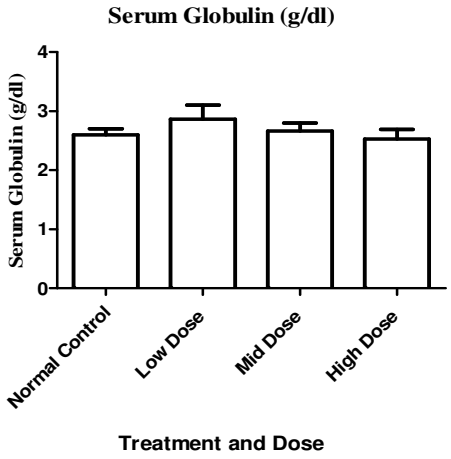
No significant difference was observed in Total Protein values of treatment groups when compared with normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	0.1580	1.487	No	ns	-0.1174 to 0.4334
Normal Control vs MidDose	0.08800	0.8282	No	ns	-0.1874 to 0.3634
Normal Control vs High Dose	0.2560	2.409	No	ns	-0.01944 to 0.5314

Fig 15: One way ANOVA analysis of Serum Albumin between the treatment groups

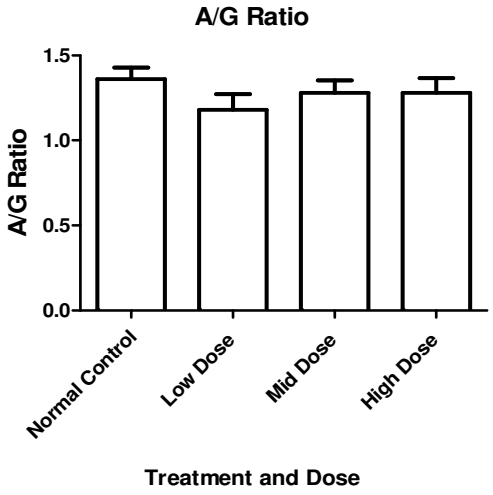
No significant difference was observed in Serum Albumin values of treatment groups as compared with normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	-0.2640	1.108	No	ns	-0.8817 to 0.3537
Normal Control vs Mid Dose	-0.06400	0.2686	No	ns	-0.6817 to 0.5537
Normal Control vs High Dose	0.07200	0.3022	No	ns	-0.5457 to 0.6897

Fig 16: One way ANOVA analysis of Serum Globulin between the treatment groups

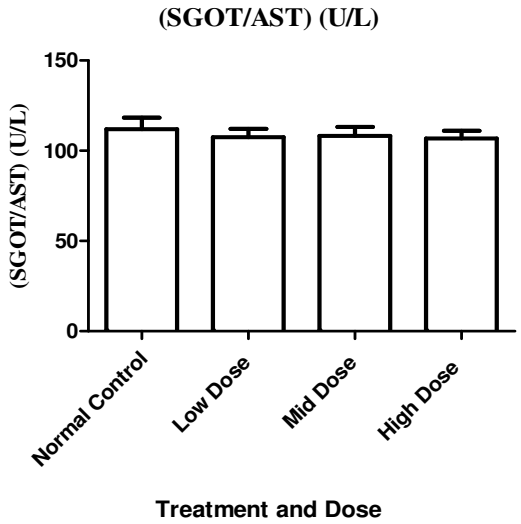
No significant difference was noticed in Serum Globulin values of treatment groups as compared with normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	0.1800	1.585	No	ns	-0.1144 to 0.4744
Normal Control vs Mid Dose	0.08000	0.7044	No	ns	-0.2144 to 0.3744
Normal Control vs High Dose	0.08000	0.7044	No	ns	-0.2144 to 0.3744

Fig 17: One way ANOVA analysis of A/G Ratio between the treatment groups

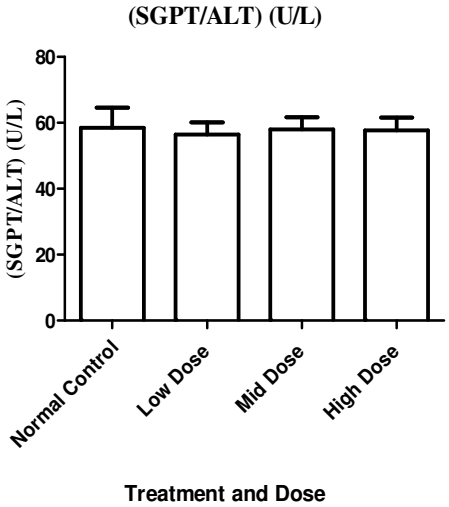
No significant change was noticed in A/G Ratio values of treatment groups when compared with normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	4.400	0.6091	No	ns	-14.33 to 23.13
Normal Control vs Mid Dose	3.600	0.4983	No	ns	-15.13 to 22.33
Normal Control vs High Dose	5.000	0.6921	No	ns	-13.73 to 23.73

Fig 18: One way ANOVA analysis of SGOT/AST between the treatment groups

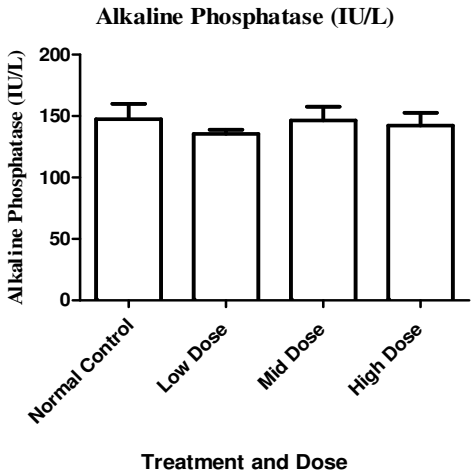
No significant change was noticed in SGOT/AST values of treatment groups when compared with normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	2.000	0.3190	No	ns	-14.26 to 18.26
Normal Control vs Mid Dose	0.4400	0.07017	No	ns	-15.82 to 16.70
Normal Control vs High Dose	0.7000	0.1116	No	ns	-15.56 to 16.96

Fig 19: One way ANOVA analysis of SGPT/ALT between the treatment groups

No significant change was noticed in SGPT/ALT values of treatment groups when compared with normal control group



Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Normal Control vs Low Dose	12.00	0.8539	No	ns	-24.43 to 48.43
Normal Control vs Mid Dose	1.000	0.07116	No	ns	-35.43 to 37.43
Normal Control vs High Dose	5.200	0.3700	No	ns	-31.23 to 41.63

Fig 20: One way ANOVA analysis of Alkaline Phosphatase between the treatments groups

No significant difference was noticed in Alkaline Phosphatase values of treatment groups when compared with normal control group

4.Discussion:

Since centuries medicinal plants are utilised for the treatment of different diseases .[7]The popularity of phytotheraphy is increased because of encourages by WHO towards relevant ethnomedicinal application to manifest the evaluation of herbal medicine in safe .[8-11]The ratification of safety and efficacy of herbal treatment by conduction of different numerous toxicity indicators emphasize by FDA &WHO [12]No abnormality were detected in the animal and no animal found to be dead in low ,middle and higher doses which is showed in **table 1** ,similarly no significant changes were noted in the behavioural pattern of the animals particularly respiration ,convulsion ,somatomotor activity ,tremor and itching .In the period of 14 days toxicity study there were no drastic changes found in food and water consumption of the animals is summarized in **table 3 and table 4**. and the body weight variation of the animals was nonsignificant is showed in

table 2 and figure 1 .It indicates the processing of protein ,carbohydrate and lipid metabolism were normal at inside the animal body because of major role played by these nutrients in various physiological function of the body [13-15].Kidney ,lungs ,heart ,and liver and spleen are the crucial organs of the human body and would be the most attacked area of any poisonous compound metabolically [16].At the end of the study all the animals were sacrificed and it was subjected to macroscopical examination .No lesions were found and organ to body weight index of mice in test group in comparison with vehicle control group were insignificant .As per the harmonized system of classification globally chemicals are splitted into five groups based on their LD50[17].The hydroalcoholic leaf extract of mesua ferrea can be put in group 5 (LD50>2000mg/kg)falling in lower toxicity classes .

In acute toxicity evaluation of M.ferrea leaf extracts ,the biological parameter are measured to evaluate the body health status .If the drug is hepatotoxic can injured the liver which results in higher level of ALT,AST and total protein levels .[18-20].Therefore no significant difference was observed in all the clinical parameters such as total bilirubin ,direct bilirubin ,Indirect bilurubiin,Serum albumin ,serum globulin,Albumin globulin ratio, aspartate amino transferase ,alanine aminotransferase ,alkaline phosphatase ,serum creatinine ,blood urea nitrogen ,was observed in this study is summarized in **table 5 and figure 2to10**.The damage of hepatocellular which results in increased permeability of cell membrane and release the amino transferase towards blood stream.[18,21,22]The standard marker for biliary tract obstruction is ALP [23].In this study there were no much difference in ALP levels in treatment group when compare to control as the ALP level is not increased but slightly decreased when compare to the control which confirming the plant is hepatoprotective .[24]

The physiological changes in animal towards to the toxic stress or environment pollutant can be determined by measuring haematological parameters which are sensitive markers .[25]There was no significant difference was observed in all the haematological parameters of treatment groups as compared to normal control group is summarized in **table 6 and figure 11-20**.

5. Conclusion:

In the bright determination of acute oral toxicity verification it is concluded that the aqueous alcoholic extracts of mesua ferrea linn is free from the lethal or deadly effect as it does not alter any much changes in haematological and clinical parameters when all the test doses of the extract were compared with normal control which was confirmed by one way analysis. .

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