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# ACUTE AND SUBACUTE TOXICITY STUDIES OF LIVAMURTHA, TRADITIONAL FORMULATION

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**Keywords:**

Traditional formulation, Toxicity study, Doses, biochemical, Histopathological, Therapeutic

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**ABSTRACT:** Livamurtha, traditional polyherbal formulation possess hepato-protective, antibacterial, and antihepatotoxic properties used for treating liver disorders. This exploratory study was designed to evaluate the acute and sub acute toxicity effects of traditional formulation in albino mice based on the OECD Guidelines. 10g of formulation ”Livamurtha” was boiled in 500ml of water for 1 hour. The decocation (Filtrate) obtained was evaporated and later were reconstituted in water for further analysis. In acute toxicity study, healthy albino mice were chosen for study with starting single dose of 5mg/kg/body weight followed by single doses of 50,300 and 2000mg/Kg/body weight in separate group of each 3 animals. The animals were observed for 14 days and its physical activities were noted. In sub toxicity study, the aqueous extract of formulation was administered orally at repeated dose of 300 and 600 mg/kg body weight for 28 days. The animals were sacrificed on the 29$^{th}$ day for further haematological, biochemical and histopathological examinations were done. In acute toxicity, no behavioural changes, mortality and toxicity was observed in mice during 14 day observational period.In sub acute toxicity study, there was no significant observational changes in the experimental animals. There is no evidence of toxicity as exhibited by regular observations, body weight, food consumption etc., No significant biochemical and histopathological changes were observed at the end of the study. Livamurtha, traditional formulation proves to be nontoxic and the therapeutic effect of it can be reached by the synergetic activity of phytoconstituents present in the formulation pharmacodynamically.

**INTRODUCTION:** The ancient system of Indian traditional medicine concentrates on a holistic approach to treat human diseases through the launch of symmetry in the different elements of human life, body, mind and soul. A deep exploration of available literature will bring about the hidden truth of medicine, the methodology of preparation from minerals, vegetables and animal origin.

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Phytochemicals are natural and non-nutritive plant bioactive chemical compounds that have protective, good antioxidant property and disease preventive properties against external stress and pathogenic attack [1]. Phytochemical constituents with therapeutic potential could be used as a single therapeutic agent or as combined formulations in drug development [2].

The separation of compounds depends on the solubility and volatilities of the constituent. The standardization of formulation involves stepwise procedure like extraction of plant materials, phytochemical screening, separation and isolation of the constituents and characterization of the isolated compounds. Traditional herbal formulation has been used throughout the world due to its

safety aspect and less-significant side effects. The quality assessment is of vital importance in order to justify their acceptability in the modern system of medicine. Our researches focused on the discovery of a precise combination of phytoactive traditional herbal formulation and assessment of its effective dosage to prevent liver injury induced by the anti-tuberculosis treatment regimen. Preparation of traditional formulation is usually done by mixing the extract of single herbs to get the synergetic effect or to prevent antagonistic effect arising from the main herb. There is a lack of proper quality control protocol for analyzing herbal materials and their formulation in the herbal and pharma industry. The various standardization parameters were designed to determine organoleptic, physical, physico-chemical properties, preliminary phytochemical screening, biological and toxicological evaluation.

The liver plays the predominant role in modification and disposition of xenobiotic, which expose it to toxic injury. The agents that cause liver injury are called hepatotoxicants. Hepatotoxicity is a dreadful condition resulting in severe metabolic disorders and may even lead to mortality [3].

Hepatic damage or chronic hepatic diseases are among the commonly occurring health problems worldwide, with liver cirrhosis and drug-induced liver injury accounting for the primary cause of death amongst the western and developing countries like India [4]. The hepatic injury is caused by ingestion or inhalation of dose-based drugs. Conventional drugs used in the drug-induced liver damage management are mostly inadequate and have serious effects.

## MATERIALS AND METHODS:

**Collection of Plants Specimen:** Traditional formulation "*Livamurtha*" consists of 7 ingredients, viz., *Curcuma longa, Terminalia chebula, Terminalia belerica, Emblica officinalis, Sphagneticola calendulacea, Phyllanthus amarus* and *Cuminum cyminum.* These plants have antibacterial, hepatoprotective, antihepatotoxic, uterine and intestine stimulating properities. These plants have been referred from the text "Gunapadam" first edition, 1936 (Siddha Materia Medica) written by Vaidya Ratnam K.S. Murugesa Mudaliar [5]. All these plants and the plant parts were procured from the field and local market. These plants were authenticated from Siddha Central Research Institute, Arumbakkam, Chennai.

**Preparation of Formulation:** The formulation was prepared with reference to acquired knowledge from the traditional vaithiyars who practice this system of medicine. All the ingredients were powdered separately, passed through 100 # sieve and then mixed together in equal proportions to get uniformly blended formulations. 10g of formulation "*Livamurtha*" was boiled in 500ml of water for 1 hour. The decocation (Filtrate) obtained was evaporated to dryness under vaccum at 50$^{o}$**C**-55$^{o}$**C** using a rotatory evaporator under reduced pressure. The yield of the preparation of formulation was 5.3g. Evaporated extracts were reconstituted in water [6].

## MATERIALS AND METHOD:

**Chemicals and Reagents:** The chemicals and solvents required for chemical evaluation were purchased commercially from E. Merck (Mumbai, India) and S.D. fine chemicals (Mumbai, India).

**Acute Toxicity Studies:** Healthy albino mice of either sex weighing 28±4g were chosen for acute toxicity studies as per the guidelines No: 420 given by the Organisation for Economic Co-operations and Development, Paris [OECD 420, 2001]. All the animals were accustomed to the laboratory environment about a week prior to the experiment. The study protocol was carried out with the approval of Institutional Animal Ethical Committee (IAEC) [NCP/IAEC/2015-16-12].

Three Albino mice were fasted overnight and the aqueous extract of formulation was given orally by intubation cannula at a starting dose of 5mg/kg/bwt. Animals were observed for a period of 2 hour, then occasionally for 4 h for severity of any toxic signs and mortality. The numbers of death animals were noted. Since no mortality was observed, this procedure was repeated for doses of 50, 300 and 2000mg/kg/b.wt in separate group of each three animals. Animals were observed continuously for 14 days for any behavioral changes, toxicity and mortality. The lethal dose $LD_{50}$ was calculated by the method of Miller and Tainter and $1/5^{th}$ of $LD_{50}$ was calculated as effective dose $ED_{50}$. The body weight of each

animal was monitored from day one before subjecting to toxicological studies till the end of 14th day. Animals were sacrified on the 15th day by cervical decapitation under ether anesthesia and the blood was collected immediately for further haematological and biochemical analysis.

After euthanizing, liver was immediately excised, washed in ice-cold saline and the weight was observed. Dissected organs were fixed in 10% buffered formalin and further histopathological analysis were performed.

**Sub Acute Toxicity Studies:** Healthy Wistar albino rat of either sex weighing 150-220g were divided into 3 groups of 6 animals each. First group serve as control received 1ml of the vehicle alone. The aqueous extract of formulation was administrated orally through cannula at repeated dose of 300 and 600mg/kg body weight for 28 days.

Behavioral, Toxic changes and mortality were monitored daily and body weight changes were recorded every 7days interval till the end of the study. Animals were sacrified on the 29th day and further haematological, biochemical and histopathological analysis were done.

**Haematological Parameters:** Total blood cell count was carried out in Beckman Coulter ACT 5 Diff Analyzer.

**Biochemical Parameters:** All biochemical parameters were performed in the LABINDIA Clinical Chemistry Analyzer.

**Statistical Analysis:** Statistical analysis was calculated by one way analysis of variance (ANOVA) followed by the Post Hoc Tukey HSD test using SPSS Software.

## RESULTS:

**Acute Toxicity Studies:** Acute toxicity studies of the drug play a crucial phase in the drug development process. In our study, *Livamurtha*, the traditional formulation, was designed for hepatoprotective activity. This drug was tested as per OECD guideline 420 and given orally as a single dose for acute toxicity evaluation.

This study was performed on albino mice of either sex at a single dose of 50, 300, 2000mg/kg body weight and was continuously monitored for the first 4 hours and 72 hours for any toxic effect after treatment period.

There were no behavioral changes and mortality observed in the experimental group. The observational changes were noted in the experimental animals compared with the normal control shown in **Table 1.**

**TABLE 1: PHYSICAL AND BEHAVIOURAL OBSERVATION OF ACUTE TOXICITY STUDY OF *LIVAMURTHA* ON EXPERIMENTAL ANIMALS**

| S. no. | Observation | Group I Control | Group II 50mg/kg | Group III 300mg/kg | Group IV 2000mg/kg |
|---|---|---|---|---|---|
| 1. | Body Weight | Normal | No change | No change | No change |
| 2. | Temperature | Normal | Normal | Normal | Normal |
| 3. | Lacrimation | No | No | No | No |
| 4. | Drowsiness | No | No | No | No |
| 5. | Salivation | No | No | No | No |
| 6. | Tremors | No | No | No | No |
| 7. | Food intake | Normal | Normal | Normal | Normal |
| 8. | Water intake | Normal | Normal | Normal | Normal |
| 9. | Convulsions | No | No | No | No |
| 10. | Eye color | No change | No change | No change | No change |
| 11. | Change in Skin | No change | No change | No change | No change |
| 12. | Rate of Respiration | Normal | Normal | Normal | Normal |
| 13. | Mortality | No | No | No | No |

The changes in the body weight of the treated and normal control were shown in **Fig. 1.** There is no significant changes in the body weight observed in the normal control and experimental group statistically ($p<0.001$). There is no significant change in the average weight of the liver, kidney, spleen and lung in both normal control and experimental groups as shown in **Fig. 2**.

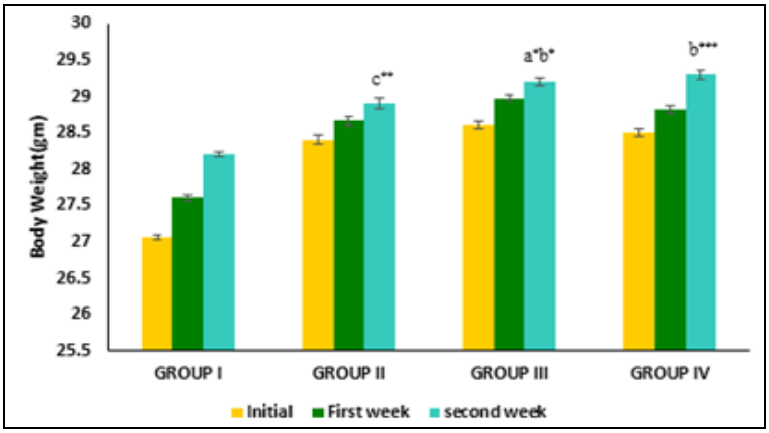


**FIG. 1: EFFECT OF *LIVAMURTHA* ON THE BODY WEIGHT OF EXPERIMENTAL MICE IN ACUTE TOXICITY STUDIES.** Each value represents the Mean±SD for six mice in each group. Comparison were made as follows: a- compared with control (Group I); b- compared with Group II; c-compared with Group III. Statistical significance: $^{***}$p<0.001;** p<0.01; *p<0.05; NS – Not significant.

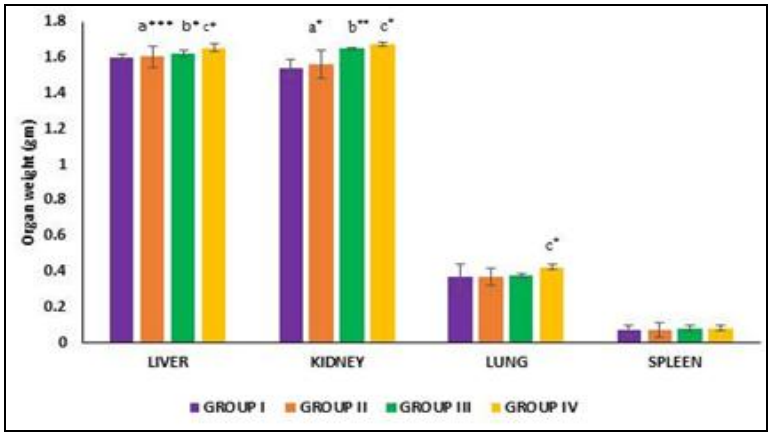


**FIG. 2: EFFECT OF *LIVAMURTHA*ON THE ORGAN WEIGHT OF EXPERIMENTAL MICE IN ACUTE TOXICITY STUDIES**. Each value represents the Mean±SD for six mice in each group. Comparison were made as follows: a- compared with control (Group I); b- compared with Group II; c-compared with Group III. Statistical significance: $^{***}$ p<0.001; $^{**}$p<0.01;$^{*}$p<0.05; NS – Not significant.

The haematological profile of the experimental and normal control was represented in **Table 2.** There was no significant difference (p<0.05) in the total RBC, WBC, and WBC differential counts of experimental animals compared to that of the control group. In the present study, there is no significant (p<0.05) change in glucose, urea, creatinine and cholesterol levels in both the experimental and normal animals, as shown in **Table 3.**

**TABLE 2: EFFECT OF *LIVAMURTHA* ON THE HAEMATOLOGICAL PROFILES OF THE EXPERIMENTAL ANIMALS IN ACUTE TOXICITY STUDY**

| Parameter | Group-1 Control | Group-II 50mg/kg | Group-III 300mg/kg | Group –IV 2000mg/kg |
|---|---|---|---|---|
| RBC x$10^6$/µl | 8.36 ± 0.56 | 8.45±0.32 | 8.71±0.28a | 9.08±0.38 |
| WBCx$10^3$/µl | 5.10±0.63 | 5.05±0.45 | 5.38±0.29a | 6.01±0.31 |
| HB(g/dL) | 11.93±0.77 | 12.61±0.73 | 12.8±0.30 | 13.33±0.37a$^{*}$ |
| PCV (%) | 43.91±1.77 | 42.58±0.95 | 43.5±1.29 | 44.78±1.55 |
| Plateletsx$10^3$ /ul | 310.16±2.9 | 318.33±8.82 | 322.67±7.39 | 331.33±1.86a$^{*}$ |
| Neutrophils (%) | 23.71±1.13 | 21.25±2.4 | 22.91±1.23 | 22.53±2.09a$^{NS}$ |
| Lymphocytes (%) | 76.41±1.05 | 74.61±2.31 | 76.75±1.22 | 77.01±1.94a$^{NS}$ |
| Eosinophils (%) | 0.2±0.03 | 0.17±0.05 | 0.18±0.09 | 0.20±0.081 |
| Monocytes (%) | 0.9±0.02 | 0.8±0.03 | 1.0±0.02 | 1.0±0.03 |
| Basophils (%) | 0.4±0.01 | 0.5±0.02 | 0.5±0.03 | 0.5±0.05 |

Each value represents the Mean ± SD for six mice in each group. Comparison was made as follows: a- compared with control (Group I); b- compared with Group II; c- compared with Group III. Statistical significance: $^{***}$p<0.001, $^{**}$p<0.01,$^{*}$p<0.05, NS- not significant.

**TABLE 3: EFFECT OF *LIVAMURTHA* ON BIOCHEMICAL PARAMETERS OF THE EXPERIMENTAL STUDIES**

| Parameter | Group-1 Control | Group-II 50mg/kg | Group-III 300mg/kg | Group –IV 2000mg/kg |
|---|---|---|---|---|
| Glucose (mg/dl) | 103.16±1.31 | 101.67±0.89 | 103.16±1.32 | 104.83±1.03a* |
| Total Protein (g/dl) | 7.18±0.10 | 7.16±0.04 | 7.21±0.05 | 7.36±0.12 |
| Total Albumin (g/dl) | 3.44±0.168 | 3.23±0.138 | 3.45±0.095 | 3.64±0.076aNS |
| Total Bilirubin (g/dl) | 0.42±0.090 | 0.41±0.01 | 0.43±0.02 | 0.441±0.02 |
| Urea (mg/dl) | 31.5±0.86 | 32.01±0.94 | 32.25±0.75 | 33.38±0.96 a* b* |
| Creatinine (mg/dl) | 0.75±0.04 | 0.72±0.07 | 0.75±0.09 | 0.76±0.05 |
| Cholesterol (mg/dl) | 120.35±1.47 | 122.65±2.09 | 124.55±2.81 | 126.45±1.58a* |

Each value represents the Mean ± SD for six mice in each group. Comparison were made as follow: a- compared with control (Group I); b- compared with Group II; c- Compared with Group III. Statistical significance: ***p<0.001, **p<0.01, *p< 0.05, NS- not significant.

There was no significant change in the level of liver enzymes AST, ALT and ALP in the experimental and normal animals, as shown in **Fig. 3.** The oxidative stress marker changes were shown in **Fig. 4.** There were no relative changes in the enzymatic antioxidant level in the tissue of treated experimental animal when compared with the normal control.

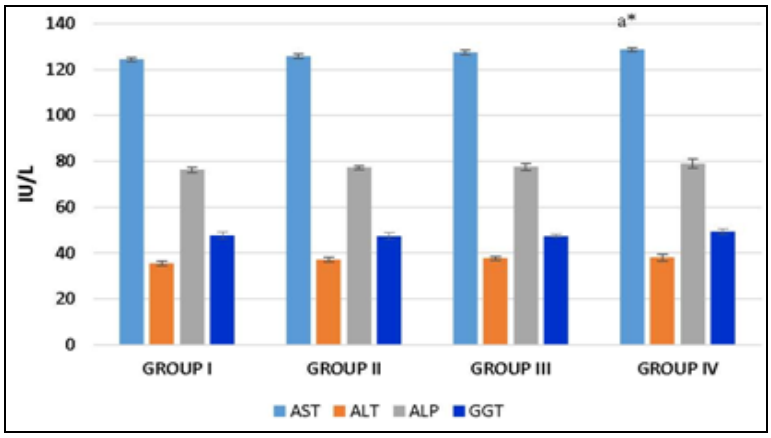


**FIG. 3: EFFECT OF *LIVAMURTHA* ON THE LIVER ENZYMES OF CONTROL AND EXPERIMENTAL RATS IN ACUTE TOXICITY STUDIES**. Each value represents the Mean±SD for six mice in each group. Comparison were made as follows: a- compared with control (Group I); b- Group II Vs GroupIII,IV; c - Group III Vs Group IV. Statistical significance: ***p<0.001; **p<0.01; * p<0.05; NS – Not significant.

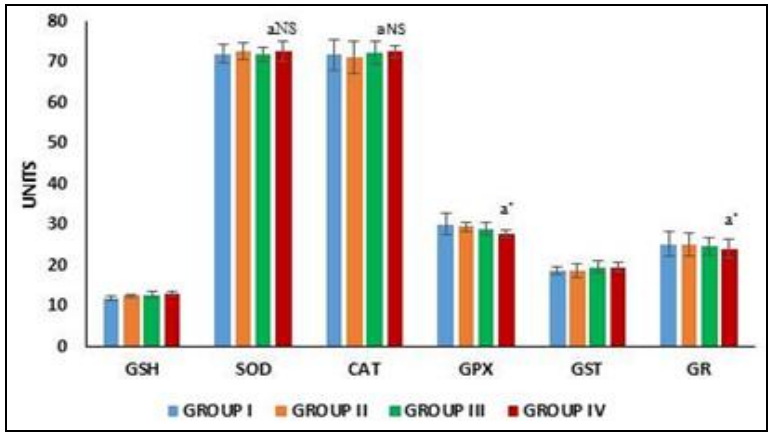


**FIG. 4: EFFECT OF *LIVAMURTHA* ON THE ANTIOXIDANT LEVELS IN THE LIVER TISSUES OF CONTROL AND EXPERIMENTAL RATS IN ACUTE TOXICITY STUDIES**. Each value represents the Mean±SD for six mice in each group. Comparison were made as follows: a- compared with control (Group I); b- Group II vs Group III, IV; c- Group III vs Group IV. SOD- U/min/mg protein, CAT-µmoles of $H_2$ $O_2$ consumed/min/mg protein, GPX-µmoles of GSH oxidized/min/mg protein, GST-nmol/min/mg protein, GR-µg of GSH utilized/min/mg protein. Statistical significance: *** p<0.001; ** p<0.01; *p<0.05; NS – Not significant.

**Histopathological Study of Acute Toxicity:** Histopathological examination of acute toxicity study shows no change in liver and kidney at a single dose of 50, 300 and 2000mg/kg body weight of aqueous extract of *Livamurtha* shown in **Fig. 5.** In **Fig. 5A** shows Normal Control (Group I) **Fig 5**B- Group II served with oral dose of 50mg/Kg body weight of Livamuratha. Photomicroscopy shows normal histology. **Fig 5C**- Group III served with oral dose of 300mg/kg body weight of Livamuratha. Photomicroscopy shows normal architecture with normal appearance of central vein, Portal tract and hepatocytes. **Fig. 5D**- Group IV served with oral dose of 2000mg/kg body weight of Livamuratha. Histopathological examination of acute toxicity study shows no change in kidney at a single dose of 50, 300 and 2000mg/kg body weight of aqueous extract of *Livamurtha* shown in **Fig. 6. Fig. 6A** shows Normal Control (Group I) **Fig. 6B** shows Group II served with oral dose of 300 mg/Kg body weight of *Livamurtha*. Photomicroscopy shows normal architecture of kidney with no inflammation or tubular atrophy. **Fig. 6C**- Group III served with oral dose of 600 mg/kg body weight of *Livamurtha*. **Fig. 6D**- Group IV served with oral dose of 2000mg/kg body weight of *Livamurtha*. Photomicroscopy shows normal architecture with normal glomerular tuft and tubular structures. No nephrotic inflammation was observed.

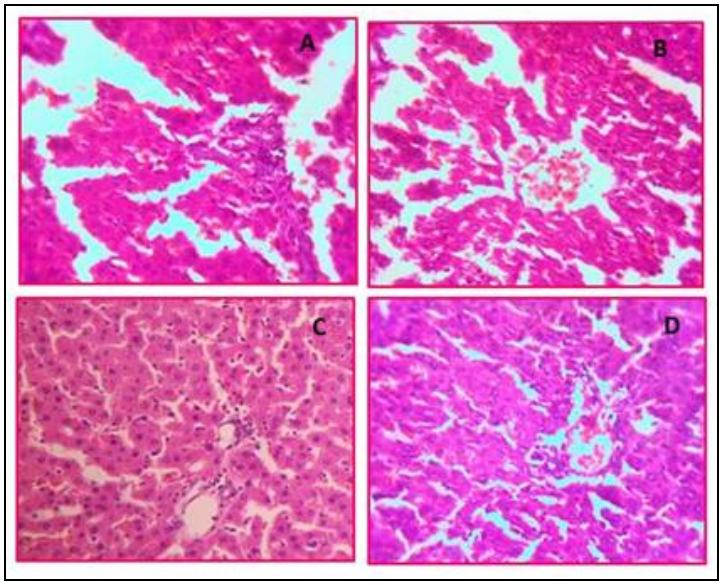


**FIG. 5: HISTOPATHOLOGICAL LIVER SECTIONS OF ACUTE TOXICITY STUDIES**. Photomicroscopy (H&E x40) liver sections of mice

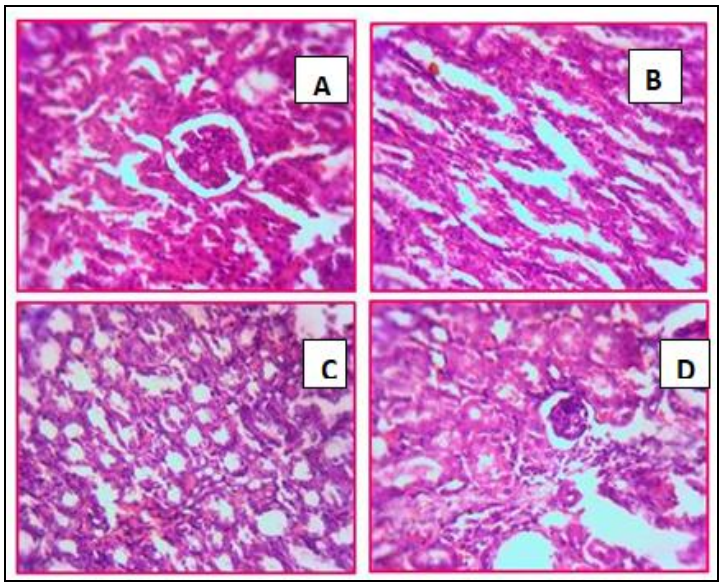


**FIG. 6: HISTOPATHOLOGICAL KIDNEY SECTIONS OF ACUTE TOXICITY STUDIES**. Photomicroscopy (H&E x40) Kidney sections of the rats.

**Subacute Toxicity Studies:** In sub-acute toxicity studies, the experiment was conducted as per OECD guidelines 420 for four weeks (28 days) with the doses of 300mg/kg/day, 600mg/kg/day and one group treated as normal control. The observational changes in the experimental animals were noted and compared with the normal control shown in **Table 4**. The body and the organ weight changes of the treated and normal control were shown in **Fig. 7** and **Fig. 8**. There is a progressive body weight gain, observed in the experimental animals.

**TABLE 4: PHYSICAL AND BEHAVIOURAL OBSERVATION OF SUB-ACUTE TOXICITY STUDY FOR EXPERIMENTAL AND NORMAL CONTROL GROUP**

| S. no. | Observation | Group I Control | Group II 300mg/kg | Group III 600mg/kg |
|---|---|---|---|---|
| 1. | Body Weight | Normal | No change | No change |
| 2. | Temperature | Normal | Normal | Normal |
| 3. | Lacrimation | No | No | No |
| 4. | Drowsiness | No | No | No |
| 5. | Tremors | No | No | No |
| 6. | Food intake | Normal | Normal | Normal |
| 7. | Water intake | Normal | Normal | Normal |
| 8. | Convulsions | No | No | No |
| 9. | Change in Skin | No change | No change | No change |
| 10. | Rate of Respiration | Normal | Normal | Normal |
| 11. | Mortality | No | No | No |

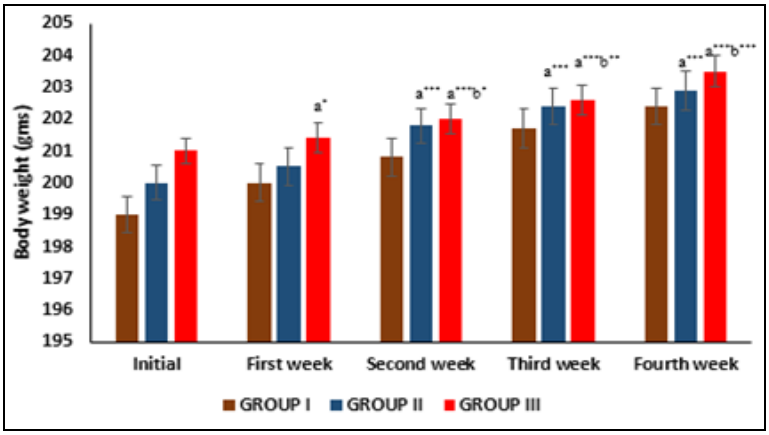


**FIG. 7: EFFECT OF *LIVAMURTHA* ON THE BODY WEIGHT OF EXPERIMENTAL RATS IN SUB-ACUTE TOXICITY STUDIES**. Each value represents the Mean±SD for six rats in each group. Comparisons were made as follows: a- compared with control (Group I); b- Group II vs Group III. Statistical significance: $^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$; NS – Not significant.

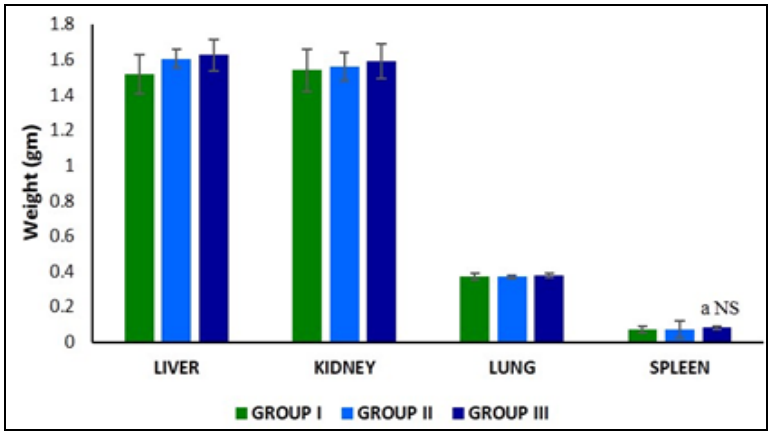


**FIG 8: EFFECT OF *LIVAMURTHA* ON ORGAN WEIGHT OF EXPERIMENTAL RATS IN SUB-ACUTE TOXICITY STUDIES**. Each value represents the Mean ± SD for six mice in each group. Comparison were made as follows: a- compared with control (Group I); b- compared with Group II. Statistical analysis was calculated by one way analysis of variance (ANOVA) followed by the Post Hoc Tukey HSD test using SPSS Software. Statistical significance: $^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$; NS – Not significant.

The hematological profile of the experimental and normal control is represented in **Table 5.** There was no significant difference in the total RBC, WBC and WBC differential count of experimental animals compared to that of the control group. The biochemical changes in the experimental animals were shown in **Table 6**.

**TABLE 5: EFFECT OF *LIVAMURTHA* ON THE HAEMATOLOGICAL PROFILES OF THE EXPERIMENTAL ANIMALS IN SUB-ACUTE TOXICITY STUDY**

| Parameter | GROUP-1 Control | GROUP-II 300mg/kg | GROUP-III 600mg/kg |
|---|---|---|---|
| RBC $x10^6$/µl | 5.48±0.53 | 6.9±0.45a*** | 8.21±0.24a*** |
| WBC$x10^3$/µl | 5.63±0.50 | 5.76±0.35a | 6.13±0.33a* |
| HB (g/dL) | 12.35±0.66 | 12.8±0.81a** | 13.5±0.68a*** |
| PCV (%) | 44.26±0.22 | 42.85±0.42 | 43.56±1.32aNS |
| Plateletsx$10^3$ /ul | 312±2.68 | 328.3±8.8 | 332.5±8.16a*** |
| Neutrophils (%) | 23.35±1.19 | 21.35±2.27aNS | 22.91±0.95 aNS |
| Lymphocytes (%) | 76.71±0.97 | 74.41±2.2 | 76.86±1.02 |
| Eosinophils (%) | 0.266±0.05 | 0.26±0.08 | 0.2±0.109 |
| Monocytes (%) | 0.8±0.02 | 0.82±0.03 | 1.0±0.02 |
| Basophils (%) | 0.5±0.01 | 0.5±0.03 | 0.5±0.06 |

Each value represents the Mean ± SD for six mice in each group. Comparison were made as follows: a- compared with control (Group I); b- compared with Group II. Statistical significance: ***p<0.001, **p<0.01, *p<0.05, NS- not significant.

**TABLE 6: EFFECT OF *LIVAMURTHA* ON BIOCHEMICAL PARAMETERS OF THE EXPERIMENTAL RATS IN THE SUB-ACUTE TOXICITY STUDIES**

| Parameter | GROUP-1 Control | GROUP-II 300mg/kg | GROUP-III 600mg/kg |
|---|---|---|---|
| Glucose (mg/dl) | 97.5±0.39 | 96.83±1.1 | 98.66±2.42b* |
| Total Protein (g/dl) | 7.1±0.14 | 7.16±0.04 | 7.24±0.04 |
| Total Albumin (g/dl) | 3.52±0.14 | 3.51±0.17 | 3.82±0.09a* |
| Total Bilirubin (g/dl) | 0.418±0.03 | 0.413±0.01 | 0.42±0.02 |
| Urea (mg/dl) | 31.45±0.59 | 31.08±0.46 | 32.18±0.80 |
| Creatinine (mg/dl) | 0.78±0.09 | 0.75±0.05 | 0.72±0.07 |
| Cholesterol (mg/dl) | 120.1±3.46 | 123.45±1.49 | 124.41±2.72 |

Each value represents the Mean ± SD for six mice in each group. Comparison were made as follows: a- compared with control (Group I); b- compared with Group II. Statistical significance: ***p<0.001, **p<0.01, *p<0.05, NS- not significant.

There was no significant change in the level of liver enzymes AST, ALT and ALP in the experimental and normal animals, as shown in **Fig. 9.** The oxidative stress marker changes were shown in **Fig. 10.** There were no relative changes in the enzymatic antioxidant level in the tissue of treated experimental animal when compared with the normal control.

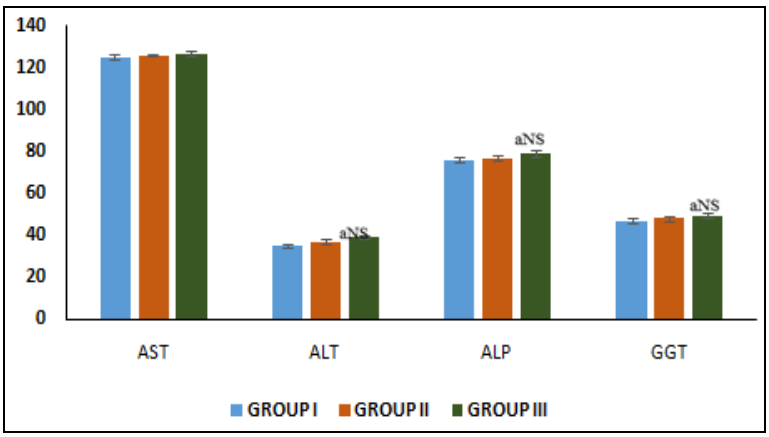


**FIG 9: EFFECT OF *LIVAMURTHA* ON THE LIVER ENZYMES OF CONTROL AND EXPERIMENTAL TOXICITY STUDIES IN THE SUB-ACUTE TOXICITY STUDIES**. Each value represents the Mean±SD for six rats in each group. Comparison were made as follows: a- compared with control (Group I); b- Group II Vs GroupIII,IV; c - Group III Vs Group IV. Statistical significance: ***p<0.001; **p<0.01; * p<0.05; NS – Not significant.

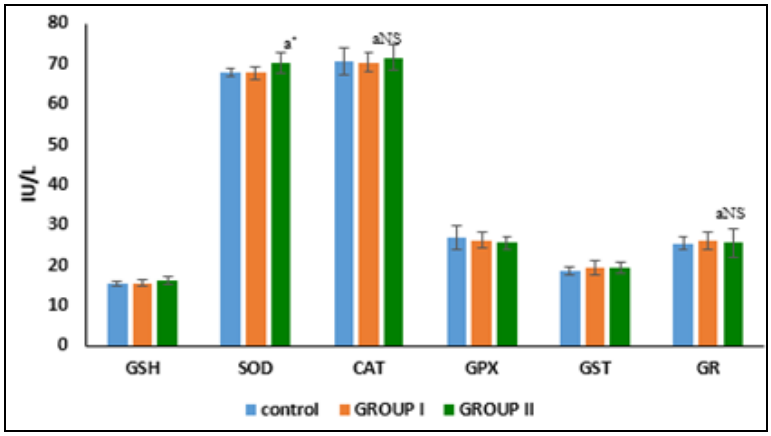


**FIG 10: EFFECT OF LIVAMURTHA ON THE ANTIOXIDANT LEVELS OF CONTROL AND EXPERIMENTAL RATS IN SUB-ACUTE TOXICITY STUDIES**. Each value represents the Mean±SD for six mice in each group. Comparisons were made as follows: a- compared with control (Group I); b- Group II vs GroupIII, IV; c- Group III vs Group IV. Statistical significance: $^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$; NS – Not significant.

**Histopathological Study of Sub Acute Toxicity:** Histopathological findings of liver and Kidney sections from the experimental groups of animals treated with the 300 and 600mg/kg b.w of aqueous extract of *Livamurtha* clearly provided evidence for the subacute toxicity study of *Livamurtha* shown in **Fig. 11** and **Fig. 12. Fig. 11A** shows Normal Control (Group I) **Fig. 11B** - Group II served with oral dose of 300 mg/Kg body weight of *Livamurtha*. **Fig 11C**- Group III served with oral dose of 600 mg/kg body weight of *Livamurtha*. Photomicroscopy shows normal architecture with no hepatocyte necrosis. **Fig. 12A** shows A- Normal Control (Group I) **Fig 12 B** - Group II served with oral dose of 300 mg/Kg body weight of *Livamurtha*. **Fig. 12C** shows Group III served with oral dose of 600 mg/kg body weight of *Livamurtha*. Photomicroscopy shows normal architecture with no inflammation or tubular atrophy.

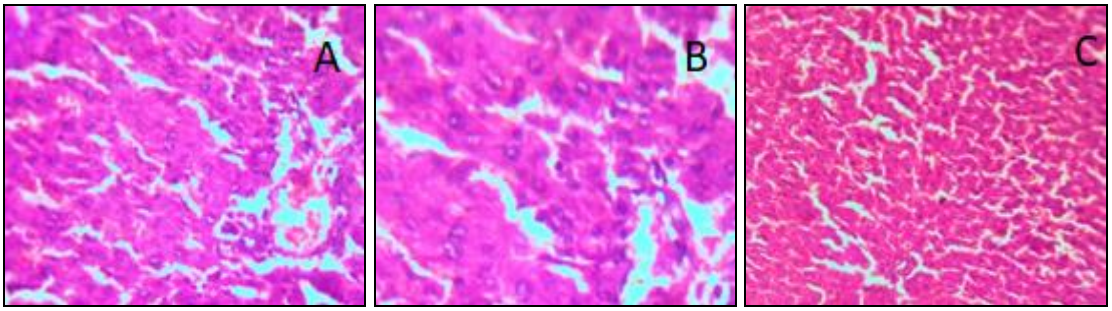


**FIG. 11: HISTOPATHOLOGICAL LIVER SECTIONS OF SUB-ACUTE TOXICITY STUDIES.** Photomicroscopy (H&E x40) liver sections of the rats.

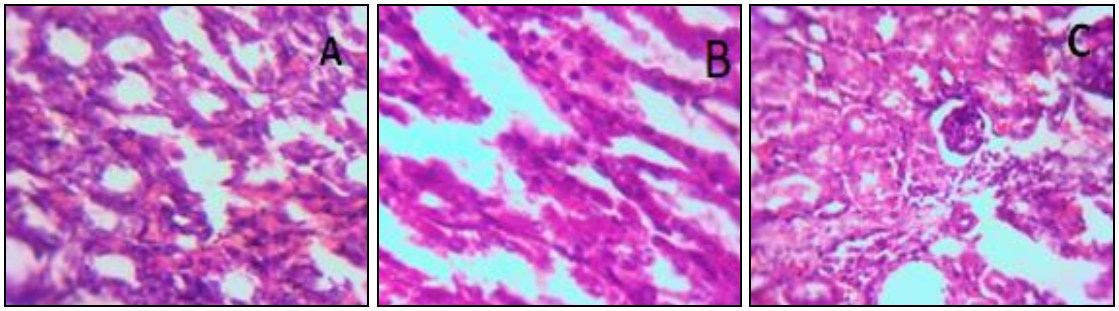


**FIG. 12: HISTOPATHOLOGICAL KIDNEY SECTIONS OF SUB-ACUTE TOXICITY STUDIES**. Photomicroscopy (H&E x40) Kidney sections of the rats.

**DISCUSSION:** Recently, there is a vast development in the field of alternative medicine, which paves the path for demand in clinical therapy. Plant origin formulation is known to play a pivotal role in the management of various diseases, which leads to the discovery of many

combinational formulations as an alternative to allopathic medicine in the modern world. However, there is a lack of proven scientific evidence on the toxicity and adverse effect of these treatments. The safety and efficacy of traditional formulation are mandatory before subjecting to clinical trial, thereby it forgoes to the toxicological evaluation of drug in animals. Therefore, the present research was performed to evaluate the *Livamurtha* for toxicity and to fix the dose for therapeutic purpose.

In the acute toxicity studies, the animals were observed for 14 days by recording skin damage, subcutaneous lumps, tenderness, abdominal distension, color of the eye, condition of the teeth, breathing abnormality etc.,. There was no significant change in the food and water intake of the experimental animals. The formulations seem to be safe at a dose level of 2000mg/kg body weight and LD is considered to be >2000mg/kg body weight. Literature survey shows that the decrease or increase in the body weight correlates with the accumulation of fats and physiological response to the formulation rather than the toxic effect of chemicals present in the formulation which causes decrease in appetite. The average weight of the liver was found to be normal, which indicates no toxic effect in both normal control and experimental animals.

There is a slight increase ($p<0.05$) in the hemoglobin and platelet level of the experimental group. The mild increase in hemoglobin and platelet levels falls within the normal reference range. Haematological changes plays significant role in determining the drug toxicity. There is no significant change in the RBC, which shows *Livamurtha* did not affect blood cell production. Total WBC is increased during stimulated lymphopoiesis or enhanced release of lymphocytes from lymph myeloid tissue; this may be due to tissue damage caused by toxic substances present in the formulation. In our study, *Livamurtha* does not alter the hematological profile of animals.

The *Embelica officinales* present in the formulation acts strongly on dyslipidemia associated with hypercholesterolemia, thereby reducing triglycerides (TG) and cholesterol [7]. Increase or decrease in the liver marker enzymes indicates hepatocellular damage. ALP is the main predictor in the diagnosis of liver and gall bladder function and a standard marker in the biliary tract obstructions. GGT is a biliary enzyme whose activity increased in the cholestatic liver disease. There was no significant difference in GGT values in the experimental and control animals. Impaired bile flow and biliary epithelial necrosis can lead to increase in GGT activity. This formulation does not affect the bile secretion and bile flow, thereby maintaining the enzyme activity.

The liver showed normal histology **Fig. 5**. Photomicroscopy shows normal architecture with no portal inflammation and cytoplasmic inclusions. There was no congestion in portal vein and intertubular blood vessels in the experimental mice. No cytoplasmic inclusions and portal inflammation were seen. Normal Control hepatocytes showed normal polygonal with oval-shaped nuclei, cytoplasm crowded with organelles, particularly rough and smooth endoplasmic reticulum, Golgi apparatus, ribosomes, mitochondria and glycogen particles.

Histopathological examinations of sections of the kidney **Fig. 6** were normal, exhibiting intact glomerular tuft and tubular structures in the treated group compared to the normal control. Photomicroscopy shows no significant change in the cortex and medulla region. No cast or inflammation in the medulla region. The Bowman capsule and the capillary tuft appeared to be normal. No nephrotic inflammation was observed.

In this study, high doses of *Livamurtha* were tolerated by the mice without producing any toxic symptoms. The phytoconstituents present in the formulation protect the animal from the toxicity. The basic principle of using crude polyherbal formulation in traditional medicine is that the toxic effect of one component is nullified by the protective effect of the other components, thus maintaining the therapeutic properties of the formulation.

In Sub acute toxicity study, the animals were observed for 28 days by recording skin damage, subcutaneous lumps, tenderness, abdominal distension, color of the eye, condition of the teeth, respiratory abnormality, etc., No toxicity or mortality was observed. In this study, no significant

behavioural changes or hair loss was observed throughout the 28 days study on daily doses of *Livamurtha*. There was no significant change in food and water intake of the experimental animals. Continuous loss of weight, approximately 10 % or more, will be considered as toxic effect of the formulation rather than any other physiological changes. There was significant increase ($p< 0.001$) in the body weight from the second week of the experimental period. The weight gain may due to improvement in the nutritional status of the animal. The average weight of the vital organs like liver, kidney were found to be normal indicating no toxic effect in both control and experimental group and there is no significant difference ($p<0.001$) in organ weight **Fig. 8**. This observation proves the safety of the formulation to other organs too. Gross examination of the organs of experimental rats revealed no pathological changes compared to the normal control.

The hematological profile of the experimental and normal control is represented in **Table 6**. There was no significant difference in the total RBC, WBC and WBC differential count of experimental animals compared to that of the control group. There was a slight increase in the haemoglobin level of the experimental group, but it falls within the normal range. Haematopoietic system is one of the most susceptible targets of toxic compound affecting bone marrow, which causes disturbance in the RBC synthesis [8]. The results show that the haemoglobin and RBC were slightly increased in experimental group III compared to the normal control. This formulation improves the Hb level, thereby preventing anemia. There was a significant increase ($p<0.05$) in WBC count in the group III but the levels of WBC falls within the normal reference range. In a toxic environment, there will be increase in accumulation of WBC cells, especially lymphocytes, leading to inflammation [9].

The biochemical changes were also observed at the end of the 28 days study. There was no significant difference ($p<0.05$) in the level of urea, creatinine and cholesterol statistically. There was a slight increase in the glucose level in group III, but its level falls within the normal range. However, the liver marker enzymes AST and ALT showed a slight increase compared to the normal control but they fall within the normal reference limit **Fig. 9**. There was no significant change in the liver marker enzyme level. These enzymes are always associated with liver cell damage, but the increase in these enzymes causes inflammation, cellular leakage and damage in the cell membrane of the hepatic cell [10, 11]. The levels of AST and ALT are known to increase during hepatotoxic conditions [12]. Detoxification of toxic compounds in the liver may cause an increase in transaminases activity. Therefore, the slight increase in hepatic enzyme after administration of the aqueous extract of the formulation may be due to the phytoconstituent which has toxic potential on hepatic cell and leads to minor injury. Moreover, these changes were not significant with toxicological and biochemical correlation. Changes in the oxidative stress marker were shown in **Fig. 10**. The level of MDA is a predictor of lipid peroxidation and oxidative damage; low level of MDA in the liver homogenate shows a reduced possibility of oxidative stress.

Oxidative stress leads not only to hepatic damage by inducing irreversible alteration of lipids, proteins and DNA contents but also modulates pathways that control normal biological functions. There was no significant ($p<0.05$) change in antioxidant enzymes of the experimental animal. Since the formulation, *Livamurtha*, contains flavonoids it enhances the antioxidant activity thereby preventing the hepatic cell from injury. Thus, natural antioxidants present in the formulation possess strong antioxidant and free radical scavenging abilities as well as anti-inflammatory activity. There were no admissible changes in the lipid peroxides of the experimentally treated group compared with the normal control. Histopathological findings of liver sections from the experimental groups of animals treated with the 300 and 600mg/kg b.w of aqueous extract of *Livamurtha* clearly provided evidence for the subacute toxicity study of *Livamurtha*.

The normal control liver showed normal hepatocellular architecture mainly consisting of normal hepatic parenchyma as shown in the **Fig. 11.** The liver appeared normal in the experimental group with normal hepatic architecture. No cytoplasmic inclusions and portal inflammation were seen. Control hepatocytes showed normal polygonal structure with oval-shaped nuclei, cytoplasm crowded with organelles, particularly

rough endoplasmic reticulum, smooth endoplasmic reticulum, Golgi apparatus, ribosomes, mitochondria and glycogen particles [13]. Histopathological examinations of sections of the kidney from rat treated with the both 300 and 600mg/kg b.w of aqueous extract of *Livamurtha* was observed to be normal exhibiting intact glomerular tuft and tubular structures. Photomicroscopy shows no significant change in the cortex and medulla region. No cast or inflammation in the medulla region. The Bowman capsule and the capillary tuft seem to be normal. No necrosis was observed, as shown in **Fig. 12.**

**CONCLUSION:** *Livamurtha* showed no toxicity with the limit dose of 2000mg/kg body weight. There was no mortality or behavioral change. The biochemical constituents, haematological parameters, liver marker enzymes was found to be within the normal range. The histopathological study of liver and kidney did not exhibit toxic symptoms. In conclusion, the therapeutic effect of the formulation can be reached by the synergetic activity of phytoconstituent present in the formulation pharmacodynamically.

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**CONFLICTS OF INTEREST:** The authors declare that there is no conflict of interest.

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