*Original Research Article*

# Assessment of the Effects of Stone Herbal Mixture Drink on Liver Parameters




## ABSTRACT

**Background:** Herbal treatments have been used for ages, across many countries and traditions to maintain health and treat diseases. Herbal medicines are sometimes seen as all-natural substitutes for traditional drugs, providing a possible means of advancing health and fitness. Investigating how herbal mixes affect various physiological characteristics, such as liver function, has drawn more attention in recent years. The stone herbal concoction under examination is made up of a number of plant-based ingredients, with unique therapeutic effects.

**Methods:** Thirty-six (36) wistar rats in total, split into six (6) groups of six (6) animals each, participated in this experimental study: In Group 1, the control group received only water and top feed; in Group 2, the standard medication (Sylimarin, 140 mg/kg body weight) plus 0.30 ml of stone herbal mixture was given; in Group 3, 0.30 ml of stone herbal mixture was given; in Group 4, 0.25 ml was given; in Group 5, 0.20 ml was given; and in Group 6, 0.15 ml was given. The rats were ethically sacrificed by cervical dislocation after receiving the daily therapy for 21 days. Each rat's brachiocephalic artery was used to draw blood, which was then placed in a lithium haparin anticoagulant bottle and examined for liver parameters.

**Results:** Group 3, 4, 5 & 6 which contains 1000mg, 850mg, 650mg and 500mg of the stone herbal mixture showed significant increase of all liver enzymes when compared to the control group. Also, the group two administered with 1000mg of the stone herbal mixture and 140 mg of the standard drug, sylimarine totally reversed toxicity in all the liver function parameters (ALT, AST, ALP, GGT, Albumin, Total protein, Bilirubin, AFP, TNF) compared to the control group one. Groups 3, 4, 5 and 6 which contains 1000mg, 850mg, 650mg and 500mg of the stone herbal mixture showed significant decrease of Albumin, bilirubin and total protein with while the 500mg dose of herbal mixture in group 6 was increased.

**Conclusion:** In conclusion, this study has unveiled valuable insights into the effects of the Stone Herbal Mixture on liver parameters in male albino Wistar rats. Our result demonstrated that the lowest dose of the stone herbal mixture which is 500 mg is tolerable with the liver as against other higher doses in this experiment.


*Keywords: Stone, Herbal, Liver, Disease, Albino.*

## 1. INTRODUCTION

Herbal treatments have been used for ages, across many countries and traditions, to maintain health and treat disease.[1] Herbal medicines are sometimes seen as all-natural substitutes for traditional drugs, providing a possible means of advancing health and fitness. Investigating how herbal mixes affect various physiological characteristics, such as liver function, has drawn more attention in recent years. The stone herbal concoction under examination is made up of a number of plant-based ingredients, each of which is said to have unique therapeutic effects.[2] Although this herbal blend has been used historically, there isn't enough solid empirical data to support its effects on several liver markers.

The liver comprises around 2% of an adult's body weight. The liver is a unique organ due to its dual blood supply from the portal vein (approximately 75%) and the hepatic artery (approximately 25%).[3,4, 5] Previous studies on the impact of herbal remedies on liver health have shown both encouraging and contradictory findings. Studies have shown that several herbal components have hepatoprotective effects, Numerous herbal medicines and substances have been demonstrated to have hepatoprotective effects, which means they can prevent or repair liver damage.[6] On the other hand, adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal.[2,7]

Traditional wisdom and anecdotal evidence point to the stone herbal mixture's hepato-protective qualities, but the scientific community lacks a thorough grasp of the mechanisms of actions underlying its effect on liver functions. There is a notable lack of empirical study on the stone herbal mixture's effects on liver parameters, despite its long history of use and general acceptance as a viable treatment for liver health. The complex interactions between the components of the herbal mixture and their possible positive or negative effects on liver parameters are also largely unexplored.[4]

The investigation into the effects of the stone herbal mixture on liver parameters in male albino wistar rats holds significant scientific and practical importance. This study's justification rests on several key factors that underscore its relevance and potential contributions. The herbal mixture under examination represents a blend of traditional knowledge and alternative medicine practices.[8] By subjecting the effects of this herbal mixture to the liver of the wistar rats to rigorous scientific investigation, this study tends to bridge the gap between anecdotal claims and empirical evidence. The outcomes have the potential to enrich our understanding of the herbal mixture's effects on liver parameters, shedding light on its mechanisms of action and its role in hepatic health.[4] The findings of this study are anticipated to advance our knowledge of how herbal remedies affect liver health. Additionally, the results might have significance for people looking for all-natural therapies to support liver function.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Materials used in this study include; Cotton wool, needle and syringe, plain bottle, Lithium heparin, methylated spirit, Gloves, micropipette, Tourniquet, automatic micropipette, pipette tips, spectrophotometer, spectrophotometric cuvette, water bathe, vortex mixer, disposable test tubes, and stone herbal mixture.

Stone Herbal mixture state was purchased from orja market in Ibadan, Oyo State, Nigeria.

Figure 1 : **herbal mixture**


### 2.2 Experimental Animals

Thirty-Six (36) Male Albino Wistar rats weighing 100-120g were used for this study. The rats were obtained from the Lead City University Ibadan, animal house, and were used for the study. The animals were allowed one week acclimatization period after which they were reweighed and housed in plastic cages with plastic bottom and

wire-mesh top, under controlled environmental conditions of temperature (28±20C), relative humidity (50±5%) and a twelve-hour light/dark cycle. The animal facility was adequately ventilated and the animals maintained regularly on the commercial rat chow. Water and Top feed were provided throughout the experimental period.

After One week of acclimatization period, rats were divided into (6) groups, the experimental control group Were administered with water and top feed, Group 2 were administered with water, top feed, (silymarin 140mg/kg body weight) and oral intubation of stone Herbal mixture while other groups were administered with water, top feed and oral intubation of stone Herbal mixture.

**2.3 Study Design**

This study was an experimental study comprising of total of Thirty-six (36) wistar rats divided into six (6) groups of six (6) animals each. The six groups were Group 1, control group fed with top feed and water only, Group 2 administered with a Standard drug (Sylimarin, 140mg/kg body weight) + 0.30 mls of stone herbal mixture and top feed, Group 3 with 0.30 ml of stone herbal mixture, Group 4 with 0.25 ml of stone herbal mixture, Group 5 with 0.20 ml of stone herbal mixture, Group 6 with 0.15 ml of stone herbal mixture. The treatment was given daily and lasted for 21 days.

**2.4 Study Site**

This study was carried out at the animal house, Department of Medical Laboratory Science, Lead City University Ibadan-Oyo state.

**2.5 Administration of Stone herbal mixture**

The herbal mixture extract was administered with the aid of oral cannula once daily to the appropriate group for the time specified for each group as well as specified dosage for each group following the experimental design.

**2.6 Ethical considerations**

All animals procedures were conducted in accordance with Lead city University- Research Ethics Committee, (LCU-REC) guidelines with the ethical approval number (LCU-REC/23/345). Measures were taking to minimize animal suffering and distress.

**2.7 Animal Sacrifice and Sample Collection**

At the end of the experiment, blood was collected into Lithium heparin anticoagulant bottle from brachiocephalic artery of each rat. The rats were immediately ethically sacrificed by cervical dislocation. At the end of the sample collection, the samples were analyzed for liver parameters.

**2.8 Biochemical assay**

All biochemical assays; Aspartate Aminotransferase, Alanine Aminotransferase, Alkaline Phosphatase, Gamma glutamyl-transferase activities, total protein, albumin, Tumor Necrotic Factor Alpha, Alpha feto-protein and Serum Bilirubin concentrations were carried out using standards kits, methods and an AJ-Semi-auto Biochemical Analyzer.

**2.9 Laboratory analysis**

Liver function test parameters which are;

**a) Alkaline Phosphatase (ALP)**

**Methodology:** Enzymatic Assay

**Principle:** Alkaline Phosphatase in serum catalyse the hydrolysis of p. nitrophenyl phosphate to p. nitrophenol and phosphate. p. nitrophenol is yellow coloured compound. As the reaction progresses the rate of absorbance increases which is proportional to the activity of Alkaline Phophatase in the sample. This reaction takes place in alkaline medium and in presence of magnesium ions. The change in absorbance is measured at 405 nm. [9]

**b) Total Protein (TP)**

**Methodology:** Biuret Method

**Principle:** Proteins, in an alkaline medium, bind with the cupric ions present in the biuret reagent to form a blue-violet coloured complex. The intensity of the colour formed is directly proportional to the amount of proteins present in the sample.[9]

**c) Alanine amino Transferase**

**Methodology**: Enzymatic Assay

**Principle**: ALT catalyzes the transfer of amino group between L-Alanine and a-Ketoglutarate to form pyruvate and glutamate. The pyruvate formed react with NADH in the presence of Lactate Dehydrogenase to form NAD. The rate oxidation of NADH to NAD is measured as a decrease in absorbance which proportional to the SGPT(ALT) activity in the sample.[9]

**d) Albumin**

**Methodology**: Bromocresol Green

**Principle**: Albumin is known for its ability to bind many types of organic compounds, including organic dyes. When albumin binds with Bromcresol Green (BCG) it causes a change in the absorbance maximum of BCG. This change can be measured spectrophotometrically and used to determine albumin concentration.[9]

**e) Bilirubin**

**Methodology:** Van Den Bergh

**Principle** - This method for bilirubin estimation is based on Van Den Bergh reaction. In this reaction, bilirubin reacts with diazotized sulfanilic acid to produce azobilirubin which is purple in color. Intensity of color is directly proportional to the amount of bilirubin in the serum. There are two kinds of reactions- direct reaction and indirect reaction. The direct reaction occurs in aqueous medium and is given by only water-soluble conjugated bilirubin. The reaction is fast and color develops in first few minutes only. That is why, conjugated bilirubin is also called direct bilirubin. In indirect reaction, methyl alcohol is added which solubilizes unconjugated, water insoluble bilirubin. Hence, indirect reaction gives the measure of total bilirubin (conjugated and unconjugated).

Unconjugated bilirubin = total bilirubin-conjugated bilirubin

In this way, unconjugated bilirubin can be calculated after indirect reaction. Also, unconjugated bilirubin is called indirect bilirubin.[9]

**f) Gamma Glutamyl Transferase (GGT)**

**Methodology:** Enzymatic Assay

**Principle:** GGT catalyzes the transfer of the gamma-glutamyl group from the donor substrate (L-gamma-glutamyl-3-carboxy-4-nitroanilide) to the glycylglycine acceptor to yield 3-carboxy-4-nitroaniline. The rate of the absorbance increase at 412 nm (416 nm for c 4000 and c 16000) is directly proportional to the GGT in the sample. The GGT procedure is a modification of the method described by Theodorsen et al. methodology: L-Gamma-glutamyl-3-carboxy-4-nitroanilide Substrate.[9]

g) **Aspartate Amino Transferase (AST)**

**Methodology**: Enzymatic Assay

**Principle**: Aspartate aminotransferase (AST)catalyzes the transfer of the amino group from L-ketoglutarate to yield oxaloacetate and L-glutarate. Molate dehydrogenase (MDH) catalyzes the reduction of oxaloacetate with simultaneous oxidation of NAD+ to NAD. The resulting rate of decreasing absorbance at 340nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.[9]

**h) Alpha feto-protein (AFP)**

**Methodology: Immunoassay**

**Assay Principle**: Diazyme's α-Fetoprotein Assay is based on a latex enhanced immunoturbidimetric assay. AFP in the sample binds to the specific anti-AFP antibodies, which are coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of AFP in the sample. The instrument calculates the AFP concentration of a patient specimen by interpolation of the obtained signal of a 6-point calibration curve.[9]

i) **Tumor Necrotic Factor (TNF alpha)**

**Methodology: Immunoassay**

**Principle of the Assay:** The Human TNF Alpha (TNF-α) ELISA employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TNF-α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNF-α present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for TNF-α is added to the wells and binds to the combination of capture antibody- TNF-α in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A colored product is formed in proportion to the amount of TNF-α present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven TNF-α standard dilutions and TNF-α sample concentration determined.[9]

**2.11 Statistical Analysis**

Data obtained was expressed as mean ± SEM and analysis was done Statistical package for Social Scientists (SPSS version 21.0). Values at $p<0.05$ were considered significant in comparison with appropriate control.

## 3. RESULTS

Group 3, 4, 5 & 6 which contains 1000mg, 850mg, 650mg and 500mg of the stone herbal mixture showed significant increase of all liver enzymes when compared to the control group. Also, the group two administered with 1000mg of the stone herbal mixture and 140 mg of the standard drug, sylimarine totally reversed toxicity in all the liver function parameters (ALT, AST, ALP, GGT, Albumin, Total protein, Bilirubin, AFP, TNF) compared to the control group one. Groups 3, 4, 5 and 6 which contains 1000mg, 850mg, 650mg and 500mg of the stone herbal mixture showed significant decrease of Albumin, bilirubin and total protein with while the 500mg dose of herbal mixture in group 6 was increased.

**Table 1: Comparison of mean ± SD of liver function parameters in the groups**

| Liver | Group 1 n-6 | Group 2 n-6 | Group 3 n-6 | Group 4 n-6 | Group 5 n-6 | Group 6 n-6 | NR |
|---|---|---|---|---|---|---|---|
| **ALT** | 17.1±2.6[a] | 17.0±2.4[a] | 55.2±3.4[b] | 41.7±10.6[c] | 42.5±1.9[c] | 34.2±3.1 | 10-40 |
| **AST** | 16.7±2.8[a] | 14.5±2.1[a] | 53.8±3.1[b] | 46.0±2.4[c] | 42.5±1.9[d] | 36.2±3.4 | 10-40 |
| **GGT** | 13.3±4.5[a] | 12.0±4.4[a] | 52.7±3.3[b] | 45.5±1.0[c] | 42.3±2.1[c] | 35.3±3.6 | 5-40 |
| **Total Protein** | 6.8±0.5[a] | 6.8± .2[a] | 4.0± .5[b] | 5.9± .5[c] | 7.7±.6[d] | 8.2 ± .5[d] | 6.0-8.3 |
| **Albumin** | 4.3 ± .7[a] | 4.0±0.4[a] | 1.8±.2[b] | 2.5±.2[b] | 3.1±.2[c] | 3.3±.2[c] | 3.4-5.4 |
| **ALP** | 15.5±5.2[a] | 15.2±3.3[a] | 42.5±1.9[b] | 37.5±1.9[c] | 32.5±1.9[d] | 24.0±3.4[d] | 9-35 |
| **(TNF α)** | 18.7±2.2[a] | 18.5±1.9[a] | 47.8±1.7[b] | 42.3±2.1[c] | 36.7±1.6[d] | 17.2±2.3[a] | 15-34 |
| **Alpha feto protein** | 13.5±4.9[a] | 15.8±1.5[a] | 48.2±1.9[b] | 42.3±1.75[c] | 38.0±1.4[c] | 32.3±2.1[c] | 10-40 |
| **Total Bilirunbin** | 0.9±0.2[a] | 0.7±0.2[a] | 3.1±0.4[b] | 2.2±0.3[b] | 1.5±0.2[c] | 1.4±0.3[c] | 0.1-1.2 |
| **CB (Direct)** | 0.2± 0.1[a] | 0.2±0.1[a] | 0.2±0.1[a] | 0.4±0.1[b] | 0.2±0.1[a] | 0.3±0.1[b] | 0-0.3 |
| **UB (Indirect)** | 0.2±0.1[a] | 0.2±0.1[a] | 0.2±0.1[b] | 0.1±0.1[b] | 0.2±0.1[b] | 0.3±0.1[c] | 0-0.3 |

Key: CB - Conjugated Bilirubin; UB - Unconjugated Bilirubin
Values in a row with the same superscript are not statistically significant at p<0.05

**Table 2: Comparison of liver function parameters in Group 1 (No inducement or treatment) with each of the six treatment groups**

| Liver Parameter | Group | Mean Difference | t | P-values |
|---|---|---|---|---|
| **ALT** | Group 2 | .16667 | .210 | .842 |
| | Group 3 | -38.00000 | -23.875 | .000 |
| | Group 4 | -24.50000 | -5.290 | .003 |
| | Group 5 | -25.33333 | -19.000 | .000 |
| | Group 6 | -17.00000 | -15.105 | .000 |
| **AST** | Group 2 | 2.16667 | 3.081 | .027 |
| | Group 3 | -37.16667 | -31.857 | .000 |
| | Group 4 | -28.83333 | -33.050 | .000 |
| | Group 5 | -25.83333 | -21.620 | .000 |
| | Group 6 | -19.50000 | -9.853 | .000 |
| **GGT** | Group 2 | 1.33333 | .610 | .569 |
| | Group 3 | -39.33333 | -39.778 | .000 |
| | Group 4 | -32.16667 | -16.888 | .000 |
| | Group 5 | -29.00000 | -13.424 | .000 |
| | Group 6 | -22.00000 | -11.595 | .000 |
| **Total Protein** | Group 2 | -.03333 | -.152 | .885 |
| | Group 3 | 2.71667 | 8.132 | .000 |
| | Group 4 | .86667 | 2.744 | .041 |
| | Group 5 | -.98333 | -3.261 | .022 |
| | Group 6 | -1.43333 | -6.748 | .001 |
| **Albumin** | Group 2 | .30000 | 1.643 | .161 |

| | | | | |
|---|---|---|---|---|
| | Group 3 | 2.55000 | 9.427 | .000 |
| | Group 4 | 1.85000 | 6.315 | .001 |
| | Group 5 | 1.23333 | 3.792 | .013 |
| | Group 6 | 1.00000 | 4.038 | .010 |
| **ALP** | Group 2 | .33333 | .141 | .893 |
| | Group 3 | -27.00000 | -11.917 | .000 |
| | Group 4 | -22.00000 | -8.521 | .000 |
| | Group 5 | -17.00000 | -7.316 | .001 |
| | Group 6 | -8.50000 | -2.718 | .042 |
| **TNF** | Group 2 | .16667 | .191 | .856 |
| | Group 3 | -29.16667 | -21.574 | .000 |
| | Group 4 | -23.66667 | -22.452 | .000 |
| | Group 5 | -18.00000 | -18.000 | .000 |
| | Group 6 | 1.50000 | 1.275 | .258 |
| **Alpha feto protein** | Group 2 | -2.33333 | -1.060 | .338 |
| | Group 3 | -34.66667 | -16.444 | .000 |
| | Group 4 | -28.83333 | -16.391 | .000 |
| | Group 5 | -24.50000 | -13.065 | .000 |
| | Group 6 | -18.83333 | -8.084 | .000 |
| **Total bilirubin** | Group 2 | .10000 | .628 | .557 |
| | Group 3 | -2.35000 | -9.772 | .000 |
| | Group 4 | -1.36667 | -8.300 | .000 |
| | Group 5 | -.75000 | -6.377 | .001 |
| | Group 6 | -.58333 | -3.035 | .029 |
| **Conjugated bilirubin** | Group 2 | .02000 | .272 | .799 |
| | Group 3 | .06000 | 1.000 | .374 |
| | Group 4 | .08000 | 2.138 | .099 |
| | Group 5 | .06000 | 1.500 | .208 |
| | Group 6 | -.04000 | -.784 | .477 |
| **Unconjugated bilirubin** | Group 2 | .02000 | .272 | .799 |
| | Group 3 | .06000 | 1.000 | .374 |
| | Group 4 | .08000 | 2.138 | .099 |
| | Group 5 | .06000 | 1.500 | .208 |
| | Group 6 | -.04000 | -.784 | .477 |

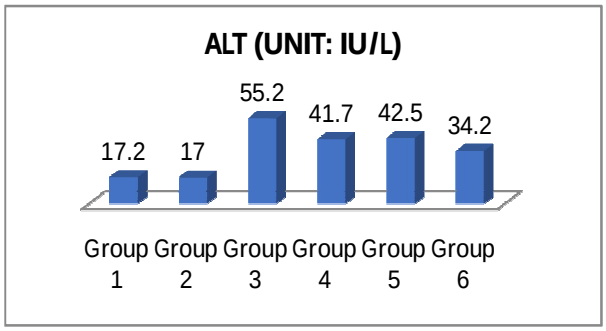


**Figure 2: Effect of Stone Herbal Mixture on Alanine aminotransferase (ALT)**

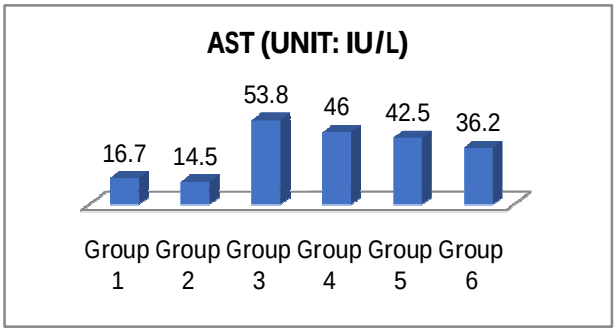


**Figure 3: Effect of Stone Herbal Mixture on Aspartate aminotransferase (AST)**

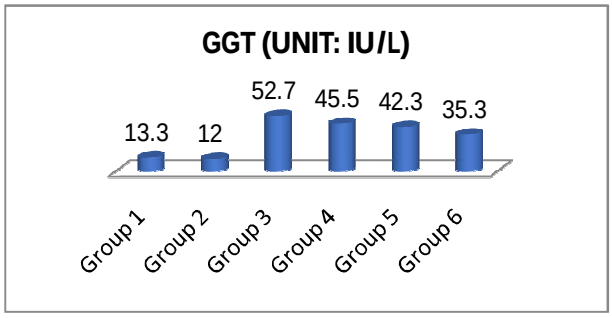


**Figure 4: Effect of Stone Herbal Mixture on Gamma Glutamyl Transferase (GGT)**

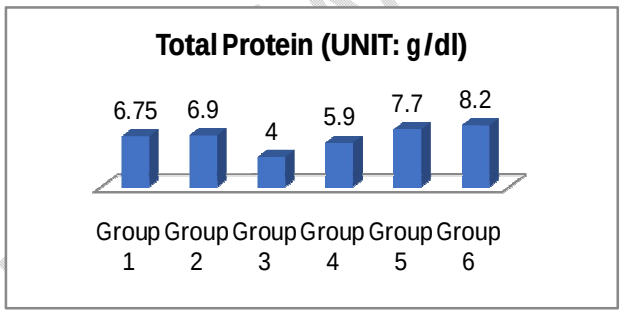


**Figure 5: Effect of Stone Herbal Mixture on Serum Total Protein**

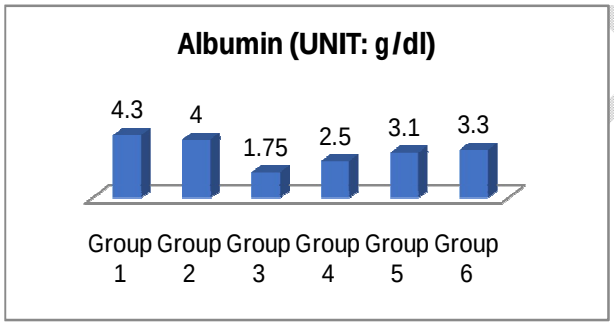


**Figure 6: Effect of Stone Herbal Mixture on Serum Albumin**

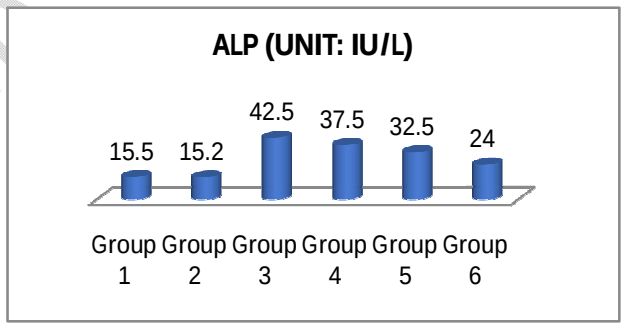


**Figure 7: Effect of Stone Herbal Mixture on Alkaline Phosphatase (ALP)**

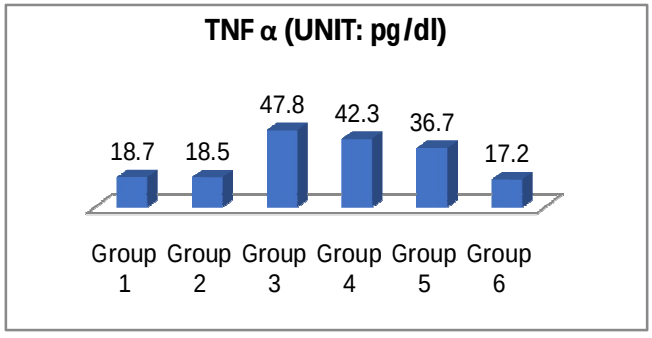


**Figure 8: Effect of Stone Herbal Mixture on Tumor Necrotic Factor Alpha (TNF α)**

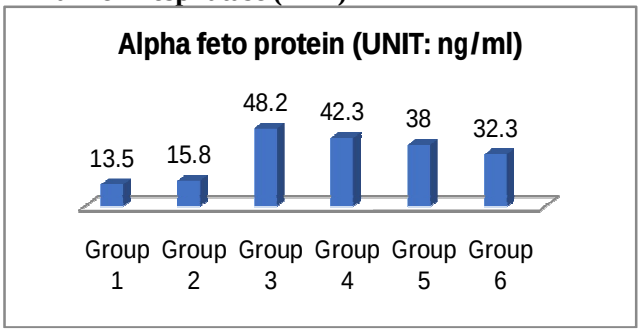


**Figure 9: Alpha feto-protein**

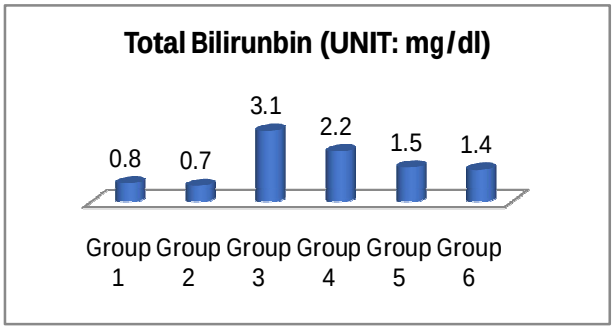


**Figure 10: Effect of Stone Herbal Mixture on Serum Total Bilirubin (STB)**

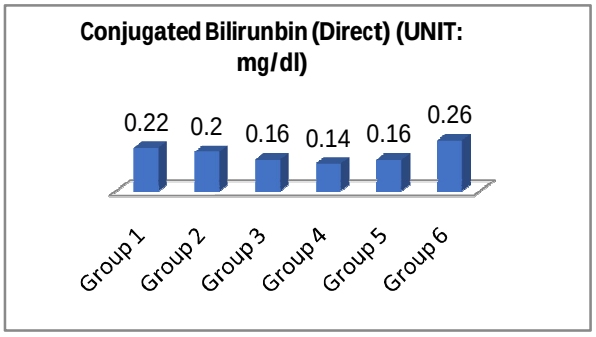


**Figure 11: Effect of Stone Herbal Mixture on Serum Conjugated (Direct) Bilirubin**

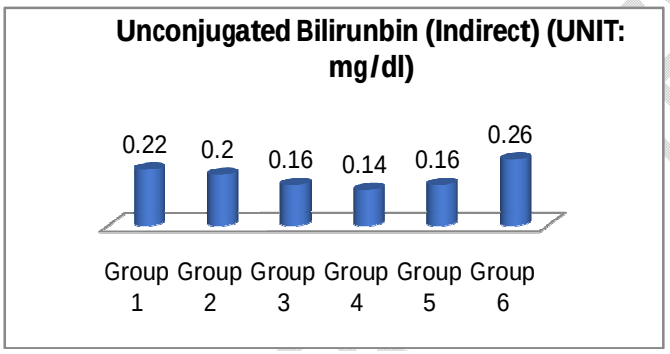


**Figure 12: Effect of Stone Herbal Mixture on Serum Unconjugated (indirect) Bilirubin**

## 4. DISCUSSION

The investigations into the effect of *Stone Herbal Mixture* on liver parameters in male albino Wistar rats yielded crucial insights into the potential impact of this herbal mixture on liver health. Findings from the analysis of liver function parameters in figure 2 revealed significant alterations in the levels of alanine aminotransferase (ALT) in some of the treatment groups; Group 3, Group 4, Group 5 and group 6 which contains 1000mg, 850mg, 650mg and 500mg of the stone herbal mixture showed significant increase of this liver enzyme ALT statistically at P<0.01 with 220 %, 142 %, 147 % and 98 % respectively when compared to the control group one. Also, the group two administered with 1000mg of the *stone herbal mixture* and 140 mg per kg body weight of the standard drug, sylimarine totally reversed toxicity in all the liver function parameters (ALT, AST, ALP, GGT, Total protein, Albumin, TNF-α, Alpha Feto Protein and Bilirubin) compared to the control group one. In the same vein, the levels of aspartate aminotransferase (AST) in the treatment groups 3, 4, 5 and 6 which contains 1000mg, 850mg, 650mg and 500mg of the stone herbal mixture showed significant increase of this liver enzyme AST statistically at P<0.05 with percentage of 222%, 175%, 154 % and 116 % respectively when compared to the control group one. The consistent elevation of ALT and AST in response to the increasing concentrations of the herbal mixture hints at its potential hepatotoxic effects, echoing previous research on the adverse effects of various compounds on the liver when in excessive amount. These elevations suggest potential liver damage or stress.[4] The increase in ALT and AST levels aligns with numerous studies that have demonstrated the utility of these markers in assessing liver health. The results is in agreement with the study carried out by Cairney[10], which supports the claim that the herbal plant Piper methysticumat causes hepatoxicity by elevating the liver enzymes.[2,11]

Figure 4 revealed in a dose dependent manner Gamma glutamly transferase was significant in the treatment groups 3, 4, 5 and 6 which contains 1000mg, 850mg, 650mg and 500mg of the *stone herbal mixture* and showed significant increase of this liver enzyme GGT statistically at P<0.01 with 296%, 242%, 218% and 165 %

respectively when compared to the control group. Gamma-glutamyl transferase (GGT) is an enzyme associated with liver and bile duct function; with increasing concentrations of the herbal mixture, potentially signifying an impact on the biliary system and liver detoxification pathways.[12] This observation resonates with prior studies highlighting the role of GGT as a marker of liver injury.[13] The higher GGT levels or concentrations in this study suggest that the *stone herbal mixture* may be affecting the biliary tract system and liver detoxification pathways due to the increased concentrations of the stone herbal mixture administered. This study is also in agreement with the work of Sheikh *et al.*,[14] who discovered elevated ALP enzyme when they worked on the herbal mixtures and plant *Larrea tridentata*.[2]

Figure 5 revealed significant alterations in the Total protein in some of the treatment groups. The group two, administered with 1000mg of the *stone herbal mixture* and 140 mg per kg body weight of standard drug, sylimarin totally reversed toxicity compared to the control group one. Groups 3, 4, 5 which contains 1000mg, 850mg, 650mg of the *stone herbal mixture* showed significant decrease of total protein before a gradual increase till the lowest concentration administered statistically at $p<0.01$ with 59%, 87% and 114% respectively. However, the 500 mg dose in group 6 reversed the total protein by 121 % when compared to the control group; thus indicating the potential influences of the herbal mixture on albumin production. Previous studies have reported alterations in hepatic protein synthesis as an outcome of hepatotoxic herbs.[15] The observed changes in total protein and albumin levels further emphasize the influence of the herbal mixture on liver function parameters.

The Albumin was also altered as seen in figure.6 in some of the treatment groups (Group 3, Group 4, and Group 5). Groups 3, 4, 5 and 6 which contains 1000mg, 850mg, 650mg and 500mg of the *stone herbal mixture* showed significant decrease of Albumin with 40%, 58 %, 72 % while the 500mg dose of herbal mixture in group 6 increased the albumin with 76 %.

Figure 7 revealed in a dose dependent manner Alkaline phosphatase was decreased in some of the treatment groups (Group 3, Group 4, and Group 5) which contains 1000mg, 850mg and 650mg of the *stone herbal mixture* statistically at $p<0.01$ with percentage of 174%, 141 % and 109% when compared with the control. Interestingly, group 6 which contained 500mg of the mixture completely elevated ALP by 154 % as compared to the control group. The increase in ALP levels signifies potential hepatotoxicity or liver stress, reinforcing the need for a deeper investigation into the safety profile of the herbal mixture.

Figure 8 revealed significant alterations in the levels of Tumor Necrotic Factor (TNF). Groups 3, 4, 5 and 6 which contains 1000mg, 850mg, 650mg and 500mg of the *stone herbal mixture* in a dose dependent manner, showed significant elevations of TNF statistically at $p<0.01$ with 155%, 126%, 96% and 91 % respectively when compared to the control group. figure 9 revealed also in a dose dependent manner Alpha feto protein was significantly elevated in Groups 3, 4, 5 and 6 which contains 1000mg, 850mg, 650mg and 500mg of the *stone herbal mixture* statistically at $P<0.01$ with of 257%, 213%, 181% and 139 % respectively when compared to the control group. Both TNF α and alpha fetoprotein levels were elevated in some of the treatment groups (Groups 3, 4, and 5), suggesting an inflammatory response and potential liver damage. TNF α is known to play a significant role in liver disease progression[16] while alpha fetoprotein is considered a marker of liver disease.[5,17] The elevation of these markers in this study is in line with previous findings, indicating that the herbal mixture may induce an inflammatory response in the liver or lead to liver damage. A study carried out by Patil *et al.,* [18] attested that some herbal plants such as *Crotalaria* spp and its mixture can cause liver damage by elevating Alpha Feto protein levels[2].

Figure 10 revealed significant elevations in the Total Bilirubin of groups 3, 4 and 5 which contains 1000mg, 850mg and 650mg of the *stone herbal mixture* at $p<0.01$ with percentage of 287%, 175% and 87% respectively when compared to the control group. However, the 500mg dose of group 6 showed a decrease 25% with as compared with the control. Figure 11 and 11 revealed significant alterations in the direct and indirect bilirubin of some of the treatment groups. Groups 3, 4 and 5 which contains 1000mg, 850mg and 650mg of the stone herbal mixture showed significant decrease of direct (conjugated) bilirubin statistically at $p<0.01$ with 72%, 63% and 72% respectively while the 500mg in group 6 successfully elevated the conjugated bilirubin by 218% when compared to the control group and concomitantly decreasing the unconjugated bilirubin, indicating an effect of the herbal mixture on bilirubin even at reduced

volumes. These findings are consistent with studies reporting changes in bilirubin metabolism due to hepatotoxic herbs at concentrations that can be toxic to the liver.[19] Bilirubin metabolism is tightly regulated by the liver and alterations in these levels can be indicative of liver dysfunction.[4,12] The observed variations in total bilirubin levels suggest that the herbal mixture may influence bilirubin metabolism pathways. However, the reduction in the levels of conjugated bilirubin were within the reference limit for the treatment group 6 of 500mg in the stone herbal mixture, thus indicative of the conjugating ability of the 500mg herbal mixture.

Assessing the long-term effects of the herbal mixture on liver function is vital, as chronic exposure to herbal compounds could lead to cumulative effects. Longitudinal studies involving extended treatment periods can provide valuable insights into the potential risks and benefits associated with prolonged use of the herbal mixture.

Regulatory agencies should monitor herbal products closely and enforce quality control measures to ensure the safety and efficacy of herbal mixtures. Public awareness campaigns should also be initiated to inform the general population about the potential risks of using herbal products without medical supervision. It is crucial to educate consumers on the importance of seeking professional advice before using herbal remedies, particularly if they have underlying health conditions.

## 5. CONCLUSION

In conclusion, the lowest dose of the stone herbal mixture which is 500 mg is tolerable with the liver as against other higher doses in this experiment. However, the findings demonstrated the necessity for further investigations, comprehensive safety assessments, clinical trials, and increased regulatory oversight to ensure the safety and efficacy of herbal products like the *Stone Herbal Mixture*. Patient safety and informed decision-making should remain paramount in the utilization of herbal remedies for healthcare and wellness. While the potential therapeutic benefits of herbal mixtures are enticing, they must be weighed against the potential risks, as demonstrated in this study, to make informed choices about their usage.

Further research is essential to better comprehend the underlying mechanisms responsible for the interactions between the stone herbal mixture and liver as observed in the elevated parameter results. It is also crucial to conduct dose-response studies to establish the optimal dosage and safe range of the herbal mixture. This will enable a more precise understanding of the concentration at which the herbal mixture may exert therapeutic effects or induce liver stress. A systematic assessment of dosage-related effects will provide guidelines for both researchers and practitioners.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

Approval for the study was obtained from the Research Ethics Committee of the College of Medical Sciences, and was carried out in strict accordance with the guideline for the care and use of animals for research committee which is in line with that set by WHO [22].

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