Effect of Administration Ethanol Leaf Extract of *Terminalia Chebula* on Liver of Wister Rat

Joseph Oyepata Simeon¹, Modupe Builders², Wazis Chama Haruna³, Joseph Opeyemi Tosin⁴, Sabastine Aliyu Zubairu⁵, Musa Tabitha Lubo⁶, Moh’d A. Sadiq⁷

¹,²Department of Pharmacology, Faculty of Pharmacy, Bingham University, Nasarawa, Nigeria
³Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, Bornu State, Nigeria.
⁴Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Rivers State, Nigeria
⁵,⁶,⁷Department of Pharmacognosy, Faculty of Pharmacy, Gombe State University, Gombe State, Nigeria.

*Corresponding Author

Abstract:-

Introduction/Aim: Subacute toxicity tests are intended to evaluate the toxicity of the chemical after repeated administration and also to help in establishing doses for the longer-term subchronic studies. Plant has been one of the original and earliest source for correcting physiological changes in the humans body. *Terminalia chebula*, commonly known as black- or chebulic myrobolan, is a species of *Terminalia*, widely used in South Asia from India and Nepal east to southwest China (Yunnan), and south to Sri Lanka, Malaysia, and across Africa. It is widely used in management of various ailments. This work is aim at evaluating the toxic effect of *Terminalia chebula* on liver of wister rats.

Method: rats of either sex were selected. Group 1 received distilled water (10 ml/kg), while group 2, 3 and 4 received *Terminalia Chebula* 200, 400 and 800 mg/kg respectively. Animals were kept in standard cages and given access to the extract, water and food orally for 28 days, after which they were weighed and sacrificed. Blood was collected by cardiac puncture and taken immediately for hematological and chem pathological analysis. The liver and heart were also harvested for histological study of the effect of the plant using haematotoxylin and eosin (H&E) staining technique.

Result: There was Significant (P<0.05) decrease in RBC, HGB, MCV, while there was no significant change in the level of neutrophiles, basophiles, eosinophiles and platelets. There were also significant (P<0.05) increase in ALP, BILT and BILD. The liver showed slight vascular congestion and lymphocyte hyperplasia at 200 and 800 mg/kg dose while there was normal features for both 400 mg/kg and control.

Conclusion: *Terminalia Chebula* should be used with caution because it may have slight effect on the liver when taken for a prolong period of time.

Keywords: *Terminalia Chebula*, rats, liver, heart

I. INTRODUCTION

The body offers various reactions to foreign substances that infiltrate it¹. For the purpose of comprehensively determining what kind of effect a chemical substance has, or may have, on the body at that time, a systemic toxicity test observes and evaluates the systemic changes on an animal administered with the substance in question². Plant-derived medicines are used in all civilizations and cultures and, hence, plants have always played a key role in health care systems worldwide. In most developing countries, the indigenous modes of herbal treatment are a part of the culture and the dominant method of healing therapy. These remedies, with a considerable extent of effectiveness, are socially accepted, economically viable and, mostly, are the only available source³. Plants used in traditional medicine, therefore, have a critical role in the maintenance of health all over the world. The drugs of herbal, herbo-mineral, and animal origin have been used by the traditional healers to maintain health and treat diseases since antiquity. Such medicines are widely used in Africa and Asia, including India and China⁴. Due to the adverse side-effects, and also the development of resistance against synthetic drugs, the uses of plant-derived drugs are becoming popular in developed countries also⁵.

The liver performs the normal metabolic homeostasis of the body as well as biotransformation, detoxification and excretion of many endogenous and exogenous compounds, including pharmaceutical and environmental chemicals. Drug-induced hepatotoxicity is a major cause of iatrogenic diseases, accounting for one in 600 to one in 3500 of all hospital admissions⁶.

*Terminalia chebula* tree is about 50-80 feet tall in height⁶. It has round crown and spreading branches. The bark is dark brown with some longitudinal cracks. Leaves are ovate and elliptical, with two large glands at the top of the petiole. The flowers are monoeicous, dull white to yellow, with a strong unpleasant odor, borne in terminal spikes or short panicles. *T. chebula* is found in the Sub Himalayan tracks from Ravi eastwards to West Bengal and Assam, ascending up to the altitude of 1500 m in the Himalayas. This tree is wild in forests of Northern India, central provinces and Bengal⁷. The tree is also spread across Africa. The fruit is mild laxative, stomachic, tonic, alterative, antispasmodic⁷,⁸. It is useful in ophthalmic, hemorrhoids, dental caries, bleeding gums, ulcerated oral cavity. Its paste with water is found to be anti-inflammatory, analgesic and having purifying and healing capacity for wounds. Its decoction is used as gargle in oral ulcers, sore throat. Its powder is a good astringent dentifrice in
loose gums, bleeding and ulceration in gums\textsuperscript{9}. It is good to increase appetite, digestive aid, liver stimulant, stomachic, gastrointestinal prokinetic agent, and mild laxative. The powder of \textit{T. chebula} fruits has been used in chronic diarrhea. It is used in nervous weakness, nervous irritability. It promotes the receiving power of five senses. It is adjuvant in hemorrhages due to its astringent nature and good for chronic cough, chorizo, sore throat as well as asthma. \textit{Terminalia chebula} is the main ingredient in the Ayurvedic formulation \textit{Triphala} which is used for kidney and liver dysfunctions. The dried fruit is also used in Ayurveda as a purported antitussive, cardiotonic, homeostatic, diuretic, and laxative. The aim of this experiment is to evaluate the effect of ethanol extract of \textit{Terminalia chebula} on the liver of wister rat.

\section*{II. MATERIALS AND METHOD}

\textit{Animals}

Male and female Wister rats were obtained from Bingham University, Animal House. They were maintained on standard animal pellets and given water \textit{ad libitum}. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee of Bingham University.

\textit{Plant collection}

Fresh leaves of \textit{Terminalia chebula} were collected from its natural habitat from nearby Karu village, Nasarawa State, Nigeria. The plant was authenticated from Department of Botany, Bingham University, Nasarawa State, Nigeria.

\textit{Plant extraction}

The leaves were shadow dried for two weeks. The dried plant material was further reduced into small pieces and pulverized. The powdered material was macerated in 70\% ethanol. The liquid filtrates were concentrated and evaporated to dryness at 40°C in \textit{vacuum} using rotary evaporator. The ethanol extract was stored at -4°C until used.

\textit{Animal study}

Twenty four (24) rats of either sex (125-300g) were selected and randomized into four groups of six rats per group. Group 1 served as the control and received normal saline (10ml/kg) while the rats in groups 2, 3 and 4 were giving 200, 400, and 800 mg/kg of extract respectively. The weights of the rats were recorded at the beginning of the experiment and at weekly intervals. The first day of dosing was taken as D\textsubscript{0} while the day of sacrifice was designated as D\textsubscript{29}.

\textit{Haematological analysis}

The rats were sacrificed on the 29\textsuperscript{th} day of experiment. Blood samples were collected via cardiac puncture. The blood was collected into sample bottles containing EDTA for hematological analysis such as Hemoglobin concentration, white blood cell counts (WBC), differentials (neutrophils, eosinophils, basophils, lymphocyte and monocyte), red blood cell count (RBC), platelets and hemoglobin (Hb) concentration using automated Haematology machine (Cell-Dyn, Abbott, USA).

\textit{Biochemical analysis}

A Portion of the blood was collected used to estimate biochemical parameters including liver enzymes: alanine amino transaminase(ALT), aspartate amino transaminase (AST), alkaline phosphatase (ALP),albumin (ALB), total protein (TP), conjugated bilirubin (BILD), unconjugated bilirubin(BILT) using a photoelectric colorimeter.

\textit{Histopathology:}

Tissues collected were preserved in 10\% formal saline solution. Small block of the tissues were taken from liver and fixed in Bouin’s fluid for 16 to 24hours. Tissue were slices and processed according to the method described by (Lison, 1960) and stained with haemotoxylin and eosin.

\section*{III. RESULTS}

\textit{Effect of sub-acute oral administration of Terminalia chebula on hematological parameters in rats}

\textit{Terminalia chebula} caused significant (p<0.05) decrease in the level of red blood cell, hemoglobin, platelet etc. and significantly (p<0.05) caused an increase in mean corpuscular hemoglobin concentration in the rats at the dose level of 400 mg/kg compared to the control. The level of basophiles, neutrophiles, eosinophils and lymphocytes were however not significantly (p<0.05) affected by mean corpuscular hemoglobin concentration (Table 1).

\textit{Statistical analysis}

Data were expressed as the Mean ±Standard Error of the Mean (SEM). Data were analyzed statistically using one-way Analysis of Variance (ANOVA) followed by Dunnnett’s post hoc test for multiple comparisons between the control and treated groups. Values of P≤ 0.05 were considered significant.

\textit{Result}

\textit{Effect of sub-acute oral administration of Terminalia chebula on hematological parameters in rats}

\textit{Terminalia chebula} caused significant (p<0.05) decrease in the level of red blood cell, hemoglobin, platelet etc. and significantly (p<0.05) caused an increase in mean corpuscular hemoglobin concentration in the rats at the dose level of 400 mg/kg compared to the control. The level of basophiles, neutrophiles, eosinophils and lymphocytes were however not significantly (p<0.05) affected by mean corpuscular hemoglobin concentration (Table 1).

\textit{Effect of sub-acute oral administration of Terminalia chebula on hepatic indices in rats}

At 200 mg/kg dose level, \textit{Terminalia chebula} caused significant (p<0.05) decrease in BILD concentration in the treated rats while at 400 mg/kg dose level significant (p<0.05)
increase was obtained in ALP levels, BILD and BILT concentrations when compared to the control. All other parameters studied showed no significant affect (Table 2).

**Effect of sub-acute oral administration of Terminalia chebula on histology of Liver of rats**

The liver showed vascular congestion, and lymphocyte hyperplasia at 200 mg/kg and 800 mg/kg, while normal features were observed at 400 mg/kg dose and in the control.

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>Treatment (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW (10ml/kg)</td>
<td>200 mg/kg</td>
</tr>
<tr>
<td>WBC (×10^9/L)</td>
<td>9.16±0.772</td>
</tr>
<tr>
<td>RBC (×10^12/L)</td>
<td>9.23±0.32</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>15.56±0.56</td>
</tr>
<tr>
<td>HCT (g/dL)</td>
<td>57.18±2.03</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>66.45±0.93</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>19.17±0.17</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>29.17±0.17</td>
</tr>
<tr>
<td>PLT (×10^9/L)</td>
<td>620.83±52.81</td>
</tr>
<tr>
<td>LYM (%)</td>
<td>86.83±4.06</td>
</tr>
<tr>
<td>NEUT (×10^9/L)</td>
<td>11.83±3.68</td>
</tr>
<tr>
<td>EOSI (×10^9/L)</td>
<td>1.53±0.34</td>
</tr>
<tr>
<td>BASO (×10^9/L)</td>
<td>1.10±0.28</td>
</tr>
</tbody>
</table>

Data presented as Mean ± SEM: n = 6. One way ANOVA, followed by Dunnett’s post hoc for multiple comparison *significantly different from the distilled water (DW) control at p<0.05. DW = distilled water.

(WBC = white blood cells, RBC = red blood cells, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT = platelet, LYM = lymphocyte, NEUT = neutrophils, EOSI = eosinophils, BASO = basophils).

<table>
<thead>
<tr>
<th>Hepatic indices</th>
<th>Treatment (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALB (g/L)</td>
<td>DW (1ml/kg)</td>
</tr>
<tr>
<td>39.60±1.72</td>
<td>43.20±0.97</td>
</tr>
<tr>
<td>113.20±6.73</td>
<td>152.00±8.99</td>
</tr>
<tr>
<td>59.80±3.01</td>
<td>67.80±10.28</td>
</tr>
<tr>
<td>292.80±79.90</td>
<td>297.20±57.60</td>
</tr>
<tr>
<td>0.22±0.07</td>
<td>0.16±0.07*</td>
</tr>
<tr>
<td>2.14±0.50</td>
<td>2.56±0.25</td>
</tr>
<tr>
<td>79.60±3.08</td>
<td>6.07±2.71</td>
</tr>
</tbody>
</table>

Data presented as Mean ± SEM: n = 6. One way ANOVA, followed by Dunnett’s post hoc for multiple comparison *significantly different from the distilled water (DW) control at p<0.05. DW = distilled water (ALB = albumin, ALP = alanine phosphatase, ALT = alanine transaminase, BILD = unconjugated bilirubin, BILT = conjugated bilirubin, TP = total protein).
Figure 1: effect of ethanol leaf extract of *Terminalia chebula* on ALP of rat

Figure 2: effect of ethanol leaf extract of *Terminalia chebula* on unconjugated (BILD) of rat

Figure 3: effect of ethanol leaf extract of *Terminalia chebula* on conjugated (BILT) of rat
There have been reports of accidental medicinal plant poisoning and overdose. In most cases, this traditionally formulated drugs are consumed without appropriately establishing the dose that is safe for use. This has resulted in many untoward effects. Hematological parameters are useful indices that can be employed to assess the toxic potentials of plant extracts in living systems. They can also be used to explain blood relating functions of chemical compounds/plant extract. Present result showed that ethanol leaf extract of *Terminalia chebula* caused a reduction in the level of red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration which means that it can significantly reduce oxygen carrying capacity of the blood and thus cause anemia. Anemia is a condition where the blood has insufficient red blood cells to carry oxygen from the lungs to the rest of the body or not enough hemoglobin, the iron-rich protein that carries oxygen inside the red blood cells and gives blood its red color. Anemia takes several forms and may vary in severity and duration. Also reductions in packed cell volume (PCV) and red blood cell (RBC) were also observed in rats administered with the extract. This implies that *Terminalia chebula* could cause disturbances in osmoregulatory system of the blood cells and/or oxidative injury to the cell membrane. The extract could suppress the haemopoietic system. The reduction may have also occurred due to lysis of blood cells. Sule et al., 2012 also observed decrease in RBC, PCV, hemoglobin and lymphocytes in rats fed with extracts of Acalypha wilkesiana. The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies.
in immune response. The extract had no effect on white blood cell parameters, suggesting that it has no effect on the immune cells and the immune system. Hepatocyte membrane disruption is associated with membrane leakage of the hepatocyte cytosolic content which is manifested by a significant elevation of the serum marker enzymes of acute hepatocellular damage, i.e., ALT and AST, and ALP. However, ALT is the most reliable among these marker enzymes. AST is known to be present in abundance in the cardiac muscle, skeletal muscle, kidneys and testes, and ALP is abundant in the growing bone. Thus, any disease state affecting any of these extrahepatic tissues significantly elevates the serum levels of these enzymes. The plant did not cause an increase in the level ALT and AST meaning it may not membrane damage to the liver cells. There was significant increase in the serum level of ALP, unconjugated bilirubin (BILD), and conjugated bilirubin (BILT). Alkaline phosphatise (ALP) estimation is the most frequently used test to detect obstruction in the biliary system. Bilirubin is the main bile pigment in humans which, when elevated causes the yellow discoloration of the skin called jaundice. Bilirubin is formed primarily from the breakdown of a substance called heme found in red blood cells. It is taken up from the blood, processed, and then secreted into the bile by the liver. Bilirubin (unconjugated or indirect) is bound to serum albumin and transferred to the liver where it is conjugated to glucuronate by glucuronyl transferase. Conjugated (direct) bilirubin is excreted into bile. There is normally a small amount of bilirubin in the blood in healthy individuals (<17 μmol/L). Conditions which cause increased formation of bilirubin, such as destruction of red blood cells, or decrease its removal from the blood stream as in liver dysfunction, may result in an increase in the level of bilirubin in the blood. Increase in the level of ALP, BILD and BILT indicate that there was obstruction in the biliary system resulting, resulting in decrease in the clearance of bilirubin in the blood and elevation of conjugated and unconjugated bilirubin. Histology study showed slight vascular congestion and lymphocyte hyperplasia which agrees with other parameters that ethanol extract of the plant may have implication in the liver, particularly when taken for a sustained period of time.

V. CONCLUSION

Result from the study suggests that ethanol leaf extract of Terminalia chebula may be systemically toxic with tendency to biliary disease, especially jaundice.

ACKNOWLEDGEMENT

The authors wish to appreciate everyone who has contributed to the success of this study.

REFERENCES


