

**THE HISTOLOGICAL AND BIOCHEMICAL EFFECTS OF SORGHUM BICOLOR
STEM BARK ON PARACETAMOL- INDUCED LIVER DAMAGE IN WISTAR
RATS (*RATTUS NORVEGICUS*).**

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2015

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DEDICATION

To GOD Almighty for guiding my life and to my parents Mr. and Mrs. Adefemi Agbaje for their immense support

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List of Abbreviations

Gamma - aminobutyric acid	GABA
Very low - density lipoproteins	VLDL
Low - density lipoproteins	LDL
Carbon- tetrachloride	CCl ₄
Tumor necrosis factor	TNF
Interferon	IFN
Interleukin	IL
N- acetyl- p- benzoquinimine	NAPQI
Glutathione	GSH
D- galactosamine	D- GaIN
Uridine diphosphate	UDP
Reactive oxygen species	ROS
Sinusoidal endothelial cells	SECs
Hepatic stellate cells	HSC
Adenosine triphosphate	ATP
Alcoholic liver disease	ALD
Alanine aminotransferase	ALT
Aspartate aminotransferase	AST
Hepatitis A virus	HAV
Hepatitis B virus	HBV
Hepatitis B surface antigen	HBsAg
Hepatitis B core antigen	HBcAg
Hepatitis C virus	HCV
Hepatitis D virus	HDV

Hepatitis E virus	HEV
Hepatitis G virus	HGV
Enzyme - linked immunosorbent assay	ELISA
Nonalcoholic steatohepatitis	NASH
Glomerular filtrate rate	GFR
Gamma glutamyl transpeptidase	GGT
Glutathione peroxidase	GPx
Glutathione S - transferase	GSTs
Superoxide dismutase	SOD
Malonaldehyde	MDA
Polyunsaturated fatty acids	PUFA
Thiobarbituric acid reactive substances	TBARS
Nitric oxide	NO
Lecithin cholesterol acyltransferase	LCAT
Colony forming units of erythroid precursors	CFU- E
Distrene plasticizer and xylene	DPX
Livolin Forte	LIV
Essential Forte	ESF
Catalase	CAT
Curative group	CG
Prophylactic group	PG
Phosphatidylcholine	PC
Polyenylphosphatidylcholine	PPC
International unit per liter	IU/L

Silymarin

SILY

Sorghum bicolor

SB

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ABSTRACT

This study investigated the protective and ameliorative potential of aqueous extract of *Sorghum bicolor* stem bark against paracetamol-induced hepatotoxicity in rats. Thirty five adult Wistar rats weighing 150 to 200g were randomized into seven groups of five animals each. Group A (control) received distilled water of equal volume to extract (1ml), group B received 200mg/kg body weight. (bw) aqueous extract of *Sorghum bicolor* stem bark for 7days, group C received 300mg/kg bw paracetamol for 1 day, group D received 300mg/kg bw paracetamol for 1 day plus aqueous extract of *Sorghum bicolor* stem bark (200mg/kg bw) for 7 days, group E received 300mg/kg bw paracetamol for 1 day plus silymarin (100mg/kg bw), group F received 200mg/kg bw aqueous extract of *Sorghum bicolor* stem bark for 7 days plus paracetamol (300mg/kg bw) for 1 day and group G received silymarin (100mg/kg bw) and paracetamol (300mg/kg bw). All solutions were administered orally for 8 days. At the end of the administration, animals were sacrificed under chloroform anesthesia, and blood samples collected via cardiac puncture. Blood samples were used to calculate concentrations of hemoglobin, red blood cells, and white blood cells, packed cell volume, mean haemoglobin volume, mean corpuscle haematocrit, and mean corpuscle haemoglobin concentration. Sera were obtained to assay the levels of liver enzymes including aspartate amino transferase, alanine amino transferase, alkaline phosphatase, and serum bilirubin, Paraffin sections of liver were stained for histology using hematoxylin and eosin, and for histochemistry using Masson's trichome, Gordon & Sweets, and Periodic acid schiff's staining techniques. Results showed that in group C there was congestion of hepatic portal triad, sinusoids were dilated, and there was necrosis of hepatocytes nuclei. In group B that was given aqueous extract of *Sorghum bicolor* stem bark only, liver architecture was normal, group D and G showed mild portal triad congestion, mild dilated sinusoids, presence of massive inflammatory cells, hepatocytes showed dysplastic changes, Group E and F showed mild portal triad congestion, mild dilated sinusoids, reduced inflammatory cells, and dysplastic changes. Group C showed significant increase in the activities of aspartate aminotransferase, alanine aminotransferase, bilirubin and alkaline phosphatase compared to

each of group A and B ($p < 0.05$). However, the level of these enzymes significantly reduced in groups D, E, F and G compared to group C ($p < 0.05$). In comparison with the control group, there was no significant increase in the value of packed cell volume value across the experimental groups, but there was significant difference when compared with the extract only group ($p < 0.05$). There was a significant increase in the hemoglobin and red blood cells of extract only group, when compare to the paracetamol-induced toxicity group ($p < 0.05$), but there was no significant difference when compared to the other experimental groups and control. Other hematological parameters showed no significant difference across the all the groups ($p > 0.05$).

The results of this study indicated that toxic dose of paracetamol was hepatotoxic, and *Sorghum bicolor* stem bark extract had ameliorative and protective effect on the liver damage.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The liver is the largest gland in the body and, after the skin, the largest single organ (Moore *et al.*, 2010). It weighs between 1.5 kg and 2.0 kg in the average adult human and is located in the right upper quadrant of the abdomen where it is protected by the thoracic cage and diaphragm (Moore *et al.*, 2010). The blood flow to the liver is around 20% to 25% of the total cardiac output (Burt & Day, 2002). The liver receives a dual blood supply with about 30% of blood coming from the hepatic artery and 70% from the portal circulation (Burt & Day, 2002). The liver is closely associated with the small intestine, processing the nutrient-enriched venous blood that leaves the digestive tract (Moore *et al.*, 2010).

Almost all blood that enters the liver via the portal tract originates from the gastrointestinal tract as well as from the spleen, pancreas and gallbladder (Moore *et al.*, 2010). A second blood supply to the liver comes from the hepatic artery, branching directly from the celiac trunk and descending aorta (Moore *et al.*, 2010). The portal vein supplies venous blood under low pressure conditions to the liver, while the hepatic artery supplies high-pressured arterial blood. Since the capillary bed of the gastrointestinal tract already extracts most oxygen, portal venous blood has low oxygen content. Blood from the hepatic artery on the other hand, originates directly from the aorta and is, therefore, saturated with oxygen. Blood from both vessels joins in the capillary bed of the liver and leaves via central veins to the inferior cava vein.

The liver performs over 500 metabolic functions, resulting in synthesis of products that are released into the blood stream (e.g. glucose derived from glycogenesis, plasma proteins, clotting factors and urea), or that are excreted to the intestinal tract (bile) (Jeyakanathan, 2004). Also, several products are stored in liver parenchyma (e.g. glycogen, fat and fat soluble vitamins). It is involved with almost all the biochemical pathways responsible for growth, fight against disease, nutrient supply, energy provision and reproduction (Ward & Daly, 1999). The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, blood coagulation, immunomodulation, secretion of bile and storage of vitamins (Guyton & Hall, 2001).

Two major types of reactions occur in the liver in the presence of exogenous substances. The first involve chemical modification of functional groups by oxidation, reduction, hydroxylation, sulfonation and dealkylation. Various enzymes including mixed oxidases, cytochromes P-450, and the glutathione S-acyltransferases are involved in such biochemical transformations that usually lead to inactivation of drugs. This step is usually followed by conversion of the resulting metabolites into more water-soluble derivatives that are excreted in the bile or urine via coupling with glucuronate, sulfate, acetate, taurine or glycine moieties (Ram, 2001).

Hepatotoxicity is defined as an injury to the liver that is associated with impaired liver function caused by exposure to a drug or another non-infectious agent (Navarro & Senior, 2006). Liver damage inflicted by hepatotoxic agents is of grave consequences (Subramoniam & Pushpangadan, 1999). Liver ailments represent a major global health problem (Baranisrinivasan *et al.*, 2009). Liver cirrhosis is the ninth leading cause of death in the USA (Kim *et al.*, 2002). Toxic chemicals, xenobiotics, alcohol consumption, malnutrition, anaemia, medications, autoimmune disorders (Marina, 2006), viral infections (hepatitis A, B, C, D, etc.) and microbial

infections are harmful and cause damage to the hepatocytes. Chemical induced damage of animal liver, especially rodents, mimic both pathogens induced as well as chemical induced liver injury in man. Hepatotoxic chemicals cause damage to the liver cells mainly by inducing lipid peroxidation and other oxidative events (Dianzani *et al.*, 1991).

Paracetamol (N-acetyl-para-aminophenol) is discovered in 1889 and is an active metabolite of phenacetin. (Brown RA, 1968) It is widely used analgesics (pain reliever) and antipyretic (fever reducer), however, it has minimal anti-inflammatory activity compared with aspirin. (Graham, *et al.*, 2001)

The analgesic effect of paracetamol is probably dependent on the rate and amount of active drug reaching the CNS, where its analgesic effect takes place. (Piquet *et al.*, 1998) It is believed that selective inhibition of the enzyme COX-3 in the brain and spinal cord explains the effectiveness of paracetamol in relieving pain and reducing fever without having unwanted gastrointestinal side effects. (Chandrasekharan *et al.*, 2002) The fever reducing action of paracetamol was due to activity in the brain while its lack of any clinically useful anti-inflammatory action was consistent with a lack of prostaglandin inhibition peripherally in the body. (Flower, Vane, 1972) However, its mechanism of action is not fully understood but it is generally accepted that paracetamol is centrally acting drug. (Piletta *et al.*, 1991) Paracetamol is available as oral, rectal and injectable formulation. (Romsing *et al.*, 2002)

Toxicity from paracetamol is not from the drug itself but from one of its metabolites, N-acetyl-p-benzoquinoneimine (NAPQI). Paracetamol biotransformation involves conjugation with glucoronide and sulphate. A small amount of paracetamol is metabolized by mixed function oxidase enzymes to form highly reactive compound NAPQ1, which is immediately conjugated with glutathione and subsequently excreted as cysteine and mercapturic conjugates. In overdoses, large amounts of paracetamol are metabolised by oxidation because of saturation of

the sulphate conjugation pathway but once the protective intracellular glutathione stores are depleted hepatic and renal damage may ensue.

Hepatotoxicity is the most remarkable feature of paracetamol overdose. (Rumack, 2002, Matthew, 1975) Acute overdoses of paracetamol can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same; the risk is heightened by alcohol consumption. Paracetamol toxicity is the foremost cause of acute liver failure. (Walker *et al.*, 1981) Renal effects of paracetamol overdose are less commonly seen than hepatic effects. However, renal impairment may be more common than previously recognized. The overall incidence of acute renal failure in patients with paracetamol poisoning is less than 2% (Blakely, McDonald, 1995), and acute renal failure occurs in 10 to 40 % of patients with severe hepatic necrosis. (McJunkin *et al.*, 1976)

Sorghum bicolor

Sorghum bicolor is an annual grass characterized by spikelets borne in pairs. It forms an important staple in the semi-arid tropics of Africa and Asia for centuries. It is still a principal source of energy, protein, vitamins and minerals for millions of the poorest people in these regions. It is grown in some developed nations for animal feed. (Manzelli *et al.*, 2005).

It is known under a variety of names such as Great millet and guinea corn in West Africa, kafir corn in South Africa, dura in Sudan, mtama in Eastern Africa, jowar in India, kaoliang in China. In Nigeria it has a variety of names such as 'karan dafi' by the Hausa, Idoma call it 'igwu' Igbo call it 'okili' and the Yoruba's of Southern part of Nigeria call it 'Oka pupa' or 'poroporo baba' (Dalziel, 1948; Ogwumike, 2002). It is an annual or short-term perennial plant, coarse, erect, with much variability in growth characteristics; culm solid or sometimes with spaces in pith, 0.6

- 5 m tall, depending on variety and growing conditions, 5 to over 30 mm in diameter. Their leaves are broad and coarse, similar in shape to those of corn but shorter and wider. It is a plant that has been used as an antitoxicant, antiabortive, cyanogenetic, demulcent (providing relief for cough), diuretics (drugs that help in discharge of urine) and emollient (skin soften and smoothen substance). The parts that are commonly used for herbal remedies include leaves, whole plant or grains. The root is used for the treatment of malaria in southern Rhodesia; the seeds for the treatment of breast diseases and diarrhoea; the stem for tubercular swellings. In India, the plant is considered anthelmintic and insecticidal [Duke & Wain, 1981]. The seeds are used as food in brewing local beer, the corn malt and cornmeal is fermented to make a sour mash, the pith is eaten and sweet culms chewed (Watt & Brayer-Brandwijk, 1962). Sorghum has been reported to be insecticide for, and in South Africa, in combination with *Erigeron canadense* it is used for eczema (Watt & Brayer-Brandwijk, 1962). The natives of Curacao drink the leaf decoction measles, grinding the seeds with those of the calabash tree for lung ailments (Morton JF., 1981).

1.1 Objectives of the Study

The Specific objectives are to;

- 1 determine the effects of extract of sorghum bicolor stem bark on the morphology of the liver in PCM- induced liver damage in Wistar rat;
- 2 assess the effects of sorghum bicolor stem bark on the following liver enzymes aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and serum bilirubin,

assess the effect of sorghum bicolor stem bark on some haematological parameters such as packed cell volume (PCV), haemoglobin (HB) red blood cell (RBC), white blood cells