TOXICOLOGICAL PROFILES OF DIRECT ADMINISTRATION OF EXTRACT OF *Gossypium barbadense* (Linn.) LEAVES.

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APPROVAL

This research project was supervised by me and approved in accordance with the partial fulfilment of the requirements for the award of Master of Science (M.Sc.) Degree in Biochemistry, Obafemi Awolowo University, Ile - Ife, Nigeria.

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Date: . . . . . .

DEDICATION

The research work is dedicated to God and my entire family members.

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ABBREVIATIONS

APx- ascorbate peroxidase;

CAT- catalase;

PCD- programmed cell death.

ROS- reactive oxygen species

ABSTRACT

This study investigated the acute and sub-chronic toxicities of direct administration of leaves of *Gossypium barbadense* Linn. with and without addition of lime in albino mice and wistar rats respectively. This was with a view to evaluating its possible toxic effects on animals.

Fresh leaves of *G.barbadense* were crushed and squeezed to obtain direct-extract while the limed-extract was prepared from the mixture of direct-extract and *Citrus medica* juice in ratio 3:1[v/v]. The direct and limed-extract were then partitioned with ethylacetate to obtainethylacetate fractions which was used for cytotoxicity test. The direct and limed-extracts were screened for phytochemical constitutents. Acute toxicity study was carried out by standard procedure, using mice of weight range of between 19 kg and 25 kg, while in sub-chronic toxicity adult wistar rat of weight range 110 kg and 150 kg were used.The animals were treated with 250 and 500 mg/kg of direct and limed-extract of *G. barbadense,* for sub-chronic study, every other day for a period of 30 days after which the animals were sacrificed; blood was collected and liver and kidney were excised from each animal. Changes in body weight and organ weight were noted at the end of the experiment. Haematological and biochemical parameters were analyzed in the blood while histological examination was carried out on liver and kidney using standard methods. The total protein concentration in the plasma and liver homogenates were determined using standard procedure. The plasma albumin, bilirubin, urea and creatinine concentration were determined. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase activities were assayed in the liver. Cytotoxicity was carried out using *Allium cepa* with different concentrations (2.5, 5 and 10 mg/ml) of ethylacetate fraction from

direct and limed-extracts.The roots of *Allium cepa* were harvested and homogenized with normal saline to obtain supernatant for biochemical analyses.

The results indicated that the median lethal dose (LD_{50}) would be above 5000 mg/kg of body weight since no mortality was recorded in acute study. Changes in body weight were observed after 30 days of sub-chronic study. Biochemical indices of plasma and liver homogenates, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and albumin were lower in the test animals compare with the controls. In contrast, totalprotein concentrations in both plasma and liver homogenate were higher in test groups than in control.Bilirubin concentration was also higher in all the treated animals.The difference was significant at ($p<0.05$) for the animals treated with direct extract. Haematological parameters maintained nearly the same level with control in all the treated groups. Additionally, the cytotoxicity test using *Allium cepa* suggested that direct-extract and ethylacetate fractions were cytotoxic but addition of lime appeared to have lowering effects on the cytotoxicity in the treatment. However, the histological study of the liver did not reveal any damage but the kidney histology indicated partial glomerulus degeneration for animals treated with 250 mg/kg bwt in both direct- and limed-extracts.

The study concluded that both direct and limed-extract of the leaves of *G. barbadense* were not toxic on animals at 250 mg/kg and 500 mg/kg body weight. However, the ethylacetate fractions obtained from both direct- and limed-extract exhibited some degree of toxic effect.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used with considerable importance in international trade (Ebong *et al*., 2008). Also, the use of plant-derived natural compounds as part of herbal preparations as alternative sources of medicaments continues to play major roles in the general wellness of people all over the world. The African continent contains some of the richest biodiversity in the world, and abounds in plants of economic importance and plants of medicinal importance which when developed would reduce our expenditure on imported drugs to meet our health needs. Herbal-based and plant-derived products can be exploited with sustainable comparative and competitive advantage. The therapeutic action of a range of wild plants, although not scientifically proven, has been discovered by indigenous people over centuries. Developing countries are often subject to shortages of funds, medical facilities and newly developed medicine, which make them more dependent on their natural resources (Mammem and Cloete, 1996).

Most diseases could be treated with the aid of plants and it was believed that the synergistic action of additives, such as animal or insect parts, yielded even stronger or more potent medicines. However, the healing action predominantly resulted from medicinal plant compounds, since the base ingredients in the majority of medicines were of plant origin (Theunis *et al*., 1992). Although, additives are still used in traditional medication today, the plant kingdom in particular has proved to be most valuable in the treatment of ailments. Around 147 plant

families are used traditionally for medicinal purposes by many developing countries. The most prominent of these, listing over 50 plant species each, are the Fabaceae, Asteraceae, Euphorbiaceae, Rubiaceae and Orchidaceae families. The wide spread global use of the majority of these families' highlights the traditional focus on herbal plants and trees for healing purposes. An analysis of the most important medicinal plants has revealed that roughly a third of the most frequently used indigenous plants are tree species. Another third can be classified as herbaceous plants or shrubs. The rest of the spectrum is divided between rhizomatous, succulent or leafy, and bulbous plants, of which the latter contributes the smallest percentage. Most of these bulbous (monocotyledonous) plants are used for anti-inflammatory purposes, suggesting some degree of antimicrobial activity (Hutchings *et al*., 1996).

Higher plants, as sources of medicinal compounds continue to play a dominant role in maintenance of human health since antiquities. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs of the pharmaceutical industry. In developing countries, especially in rural contexts people usually turn to traditional healers when in diseased conditions and plants of ethnobotanical origin are often presented for use (Baker *et al*., 1995).

Plants are one of the most important sources of active substances with therapeutic potential to cure a variety of diseases in humans. The evaluation of pharmacological effects can be used as a strategy for discovering new drugs of plant origin. There is an ongoing world-wide revolution which is mainly premised on the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs (Alam *et al*., 2011). According to World Health Organization about 80% of the world population relies on traditional medicine for primary health care and more than 30% of the plant species have been used medicinally. However, there is limited scientific evidence regarding the safety and efficacy to support the

continued therapeutic application of these medicinal plants. Because of this renewed interest in herbal remedies and the increased use of plants extracts in food, cosmetics and pharmaceutical industries, there is a compelling need for thorough scientific safety evaluation of the medicinal plants. Laboratory animals are sensitive to toxic substances occurring in plants. Hence, the administration of the extracts in increasing amounts enables the evaluation of the acute and sub-acute toxicity limits.

Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents. Thus, many plants that are used in traditional practice are sold in a rounded urban settlement to meet the need of a public desire for panaceas which has resulted in the industrialization and large scale production of a great number of products of botanical origin widely consumed (Roja and Rao, 2000). Plants used in traditional medicine contain wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illness may be expected to have accumulated in areas where the use of plants is still of great importance (Ahsan *et al*., 2009). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga *et al*., 2005).

Traditional medicine is faced with a problem of sustainability and continuity mainly due to the loss of taxa of medicinal plants. It has been reported that, the diversity of plants is on the process of being eroded mainly due to human induced pressures. Also, it has been stated that habitat destruction and deforestation for commercial timber, encroachment by agriculture and other land uses have resulted in the loss of some thousand hectares of forest that harbor useful medicinal plants, annually over the past several decades. With the present ecological and socio-economical changes, the medicinal plants together with ethnobotanical knowledge, may disappear and thus may be lost from humanity forever (Tesfaye and Awas, 2003). These days, both human and

natural factors heavily contribute to the loss of medicinal plants, which links with the gradual displacement of indigenous knowledge associated with these plants (Sofowora, 1982).