EFFECT OF METHANOLIC EXTRACT OF *Aframomum meleguета* SEEDS ON SOME BIOCHEMICAL PARAMETERS OF THE BRAIN OF RAT TREATED WITH MONOSODIUM GLUTAMATE

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APPROVAL

This research project was supervised by me and was 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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DEDICATION

This research project is dedicated to the Almighty God and to my beloved parents.

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
ATP	Adenosine Triphosphate
CAD	Coronary Artery Disease
DTNB	Dithiobisnitro-Benzoate
DPPH	1, 1-Diphenyl-2-picrylhydrazyl
GPx	Glutathione Peroxidase
GSH	Reduced Glutathione
HDL-c	High-Density Lipoprotein Cholesterol
IC_{50}	Inhibitory Concentration
IUPAC	International Union of Pure and Applied Chemistry
LD_{50}	Lethal Dose
LDL-c	Low-Density Lipoprotein Cholesterol
MEAM	Methanolic extract of *Aframomum meleguета*
MSG	Monosodium Glutamate
NAFDAC	National Agency for Food and Drug Administration and Control
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
RBC	Red Blood Cells
RDAs	Recommended Dietary Allowances
ROS	Reactive Oxygen Species

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SOD	Superoxide Dismutase
TAC	Tannic acid Equivalent
TE	Trolox Equivalent
VLDL-c	Very Low-Density Lipoprotein Cholesterol

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ABSTRACT

The study evaluated the biochemical effects of methanolic extract of *Aframomum melegueta* seed on the brain of monosodium glutamate (MSG) neurotoxic induced rats. It also investigated the antioxidant and anti-inflammatory potential of the extract with a view to determining the efficacy of the seed extract as a remedy for management of neurotoxic disorders.

Methanolic extract of *A. melegueta* (MEAM) was prepared according to standard methods, phytochemically screened and evaluated for its anti-inflammatory and antioxidant potentials. The effect of the extract on MSG-treated rats was evaluated using thirty white albino Wister rats of either sex, which were randomly divided into six groups of five animals each. Group I served as control, Group II received MSG (2 g/kg bwt), Group III received MSG (2 g/kg bwt) and MEAM (125 mg/kg bwt) 1 hr after MSG administration, Group IV received MSG (2 g/kg bwt orally) and MEAM (250 mg/kg bwt) 1 hr after MSG administration. Also, Group V received MSG (2 g/kg bwt) and gabapentin (20 mg/kg bwt) 1 hr after MSG administration and Group VI received MEAM (175 mg/kg bwt) only. The rats were treated orally and consistently for 21 days. The behavioral activities of the animals were monitored and recorded. On the 22nd day, the rats were sacrificed after overnight fasting. Blood was collected by cardiac puncture while the whole brain was removed either for the preparation of 10%(w/v) brain homogenate or histopathological studies. The brain homogenate was used for the evaluation of brain marker enzymes, antioxidants (enzymatic and non-enzymatic), lipid profiles (brain and blood) and protein concentration was carried out using standard methods. Histopathological studies were also carried out on the brain tissue using standard techniques. Statistical analyses were carried out using GraphPad Prism 3.

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Phytochemical screening of MEAM revealed the presence of alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids. The total flavonoid content of the methanolic extract was 73.98 ± 3.08 mg/g RE (Rutin Equivalent), phenolic content was 599.04 ± 7.67 mg/g extract TAE (Tannic acid Equivalent), Vitamins C and E contents of the methanolic extract were 1.61 ± 0.04 mg/g extract and 0.12 ± 0.01 mg/g extract TE (Trolox Equivalent) respectively. The extract exhibited appreciable antioxidant potentials, with DPPH-radical scavenging of IC_{50} value of 306.84 mg/L and ascorbic acid (15.25 mg/L). The extract also exhibited appreciable anti-inflammatory properties, with percentage membrane stability ranging between 14.13% and 31.17%. The administration of MSG significantly caused reduced animal weights (6.06%), glutathione (231.10%), nitric oxide (14.29%), Vitamins C (9.89%) and E (4.85%) levels, as well as reduction of activities of glutathione peroxidase (43.86%), catalase (245.31%) and superoxide dismutase (161.54%). Increase in the activities of Na^+K^+-ATPase (61.31%), acetylcholinesterase (240.40%), glutamine synthetase (218.99%) and monoamine oxidase (32.85%), as well as increase in protein, lipid profiles and peroxidation (70.36%) levels were recorded. Histopathological observations of the brain tissues revealed that the extract protected the brain tissues from the adverse effects of MSG.

The study concluded that the extract of *A. meleguета* seed which ameliorated and reversed the adverse effects elicited by oral administration of MSG on the rats' brain in a concentration dependent manner could be used in the treatment of neurotoxic disorders.

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CHAPTER ONE

INTRODUCTION

1.1 Herbal Medicine

The word herb, as used in herbal medicine, means a plant or plant part that is used to make medicine to assist the healing process during illness and disease. An herb can be a leaf, stem, root, seed, fruit, flower and bark. These may be used in many forms, e.g. fresh, dried, cut, powdered, ointment, tincture, oil extract, or made into liquid by infusion or decoction. Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. Herbal medicine has stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The three main categories of herbs are: The food herbs which are gentle in action, have very low toxicity, and are unlikely to cause an adverse response. The second category is the "medicine herbs" which needed to be used with greater knowledge and usually for a limited period of time. The last category, the poison herbs, have strong potential for either acute or chronic toxicity and should only be utilized by clinicians who are trained to use them and clearly understand their toxicology and appropriate use (Singh *et al.*, 2014).

Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure functions including use in human therapy and scientific researches (Vasu *et al.*, 2009). However, despite the growing demand for phytotherapeutic agents (Capasso *et al.*, 2000), most medical and veterinary professionals still distrust the use of herbal medicines, due its lack of scientific evidence on efficacy and safety (Thompson, 1997). Hence the need for their scientific validation before plant-derived extracts gain wider acceptance and use. In this regard, many plants nevertheless have been scientifically proved to be effective in the control of acute and chronic

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nervous disorders. As herbal extracts are a complex mixture of compounds, the active molecules, mode of action, bioavailability, pharmacokinetics, and toxicity issues become difficult to evaluate.

1.2 Statement of the Research Problem

Monosodium glutamate (MSG) which is the sodium salt of the non-essential amino acid (glutamic acid) is commonly consumed as a flavour enhancer. Though, there is still a lot of controversy on the neurotoxic effects of MSG, studies over 30 years revealed that scientists have used MSG to induce neurodegeneration *in vivo* and *in vitro*. This study seeks to investigate both protective and ameliorative effects of methanolic extract of *Aframomum melegueta* on the selected biochemical parameters of the brain of rats treated with MSG.

1.3 Justification of the Study

Modern use of orthodox drugs in treatment, management and prevention of various forms of neurodegenerative disorders are effective though their uses have been greatly affected by their cost, side effects and adverse reaction. Relatively, plant derived medications are safer and affordable than synthetic alternatives, thereby offering therapeutic benefits. This study therefore, is designed to investigate the possibility of employing the seed extract of *Aframomum melegueta* in the treatment and management of neurodegeneration.

1.4 Specific Objectives of the Study

The specific objectives of the research are to

(a) obtain and screen the methanolic extract of *A. melegueta* seeds for phytochemicals;

(b) evaluate the antioxidant and anti-inflammatory potentials of the extract;

(c) treat rats with MSG (2 g/kg bwt) to induce neurotoxicity; and

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(d) investigate the effects of the methanolic extract of *A. meleguета* seeds on the selected biochemical parameters (brain marker enzymes, lipid profiles, enzymatic and non-enzymatic antioxidants) of the rats' brain.

1.5 Contribution to Knowledge

The study will provide baseline information on the likely neuroprotective effects of *A. meleguета* seed methanolic extracts using key biochemical parameters of the brain of rats treated with MSG. It is also envisaged that this study will provide additional information on the on-going search for natural antioxidants as alternatives to synthetic ones while providing information on the phytochemicals of methanolic extract of *A. meleguета* seed.

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