Effect of *Artocarpus heterophyllus* (Jackfruit Seed) extract on the modulation of stress in experimental mice

M. Pharm (Masters) Thesis Report

A thesis report submitted to the Department of Pharmacy in the Faculty of Allied Health Sciences at Daffodil International University in partial completion of the requirements for the masters of pharmacy degree

Submitted To

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APPROVAL

A thesis **paper "Effect of *Artocarpus heterophyllus* (Jackfruit Seed) extract on the modulation of stress in experimental mice''** submitted to the Department of Pharmacy, Faculty of Allied Health Science, Daffodil International University has been accepted as satisfactory for partial fulfillment of the requirement for the degree of the Masters of Pharmacy and approved as to its style and contents.

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Declaration

I, Mst Fateha Arefin hereby declare that I have partially fulfilled the requirements set forth by Daffodil International University for the Masters of Pharmacy degree, working under the direction of Department of Pharmacy Assistant Professor Mr. Md. Mizanur Rahman. Not a single other university or organization has ever received the investigation's findings for a degree.

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Certificate

This attests to the fact that the research results presented in the dissertation are unique and haven't been fully submitted for credit toward the awarding of any other degree or certification from an academic institution. This entire project was submitted as a thesis in order to earn a bachelor's degree in pharmacy from an undergraduate program.

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Mst Fateha Arefin

DEDICATED TO - MY FAMILY, AS WELL AS ALL OF MY RESPECTED EDUCATORS WHO HAVE CONSISTENTLY GAVEN ME SUPPORT AND ENCOURAGEMENT

Abstract

Background: Stress is a major concern for human being because it affects the person's overall wellbeing. Rather than using chemical medications to modulate the stress responses, researchers are now using nutraceuticals. **Objective:** To investigate the impact of *Artocarpus heterophyllus (jackfruit* seed) extraction on stress modulation in experimental mice in order to ascertain potential therapeutic benefits. **Materials and Method:** *Artocarpus heterophyllus* jackfruit seed was used in a 7days study to see how stress affected swiss albino mice. By supplementing the feed with cortisol, stress was created. The following parameters were measured: serum cortisol, blood glucose level, weight variation and spleen size.There were four treatments group (control, stress, an stress- standard drug group, and stress- jackfruit seed group). **Results**: When comparing the stress and stress jackfruit seed treatments, jackfruit seed had no discernible impact on any of the parameters. **Conclusion:** However, the outcome demonstrated the important imperative impact of stress.

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CHAPTER 1

INTRODUCTION

1.1General information:

Stress is an organism's reaction to unpleasant stimulus and the ensuing adaptive modifications to satisfy environmental demands. Since stress is inherently subjective, the objective measure of stress in this field of study is the organism's response to the stressor. [1]. A general requirement to maintain the steady state necessary for successful adaptation, the stress response. This entails a range of physiological or psychological responses meant to neutralise the impact of stresses and restore equilibrium [2]. Every system in the body is impacted by the global stress response. Several processes are activated in a few seconds. Characteristic processes of the stress response includes mobilization of stored energy with inhibition of subsequent energy storage and gluconeogenesis, sharpened focused attention to the perceived threat, increased cerebral perfusion rates and cerebral glucose use, enhanced cardiovascular output, and respiration, enhanced delivery of energy substrates to the muscles, inhibition of reproductive physiology and behavior, modulation of immune function, and decreased feeding and appetite [3]. Stress and emotional stimuli cause the stress hormones (glucagon, catecholamins, cortisol, and GH) to secrete more, with cortisol in particular [4-11]. Some of these hormones are diabetogenic and may contribute to the onset of diabetes during stressful situations [8].For instance, adrenaline prevents the release of insulin in both humans and animals[12], hence having a diabetogenic impact.[13]. Stress causes the hypothalamo-pituitary-adrenal (HPA) axis to react [14]. When people have chronic conditions, their weight and adrenal gland activity can occasionally rise and increase the levels of the cortisol metabolite [15-17]. Due to its permissive influence on the action of some hormones (catecholamines) and the production of other hormones (GH), cortisol[15], could lead to diabetes[13].

1.2Drug induced stress

Synthetic glucocorticoids, or GCs, such hydrocortisone are used as immunosuppressive and anti-inflammatory medications. It is frequently administered to treat a variety of illnesses[18]. Despite its many beneficial uses, hydrocortisone has been linked to a number of very harmful side effects, particularly when taken in large dosages and over extended periods of time[18]. Few molecules have been properly examined in this area, despite growing understanding that medications and chemicals might regulate the immune system through indirect processes. Elevated glucocorticoid levels are indicative of stresslike responses induced by several environmentally important substances. Similar glucocorticoid levels brought on by

psychological or physical stressors are regularly linked to the inhibition of one or more immune parameters[19]. Numerous substances and medications have the potential to severely impair immune function and lessen the host's defences against neoplasia and infectious diseases[20]. Elevation of glucocorticoids due to stress can happen even in the absence of significant immunosuppression[21] External stresses, even in the absence of increased glucocorticoid levels, can cause immunosuppression in animals with adrenalectomy[22]. Your body might create cortisol in response to stress after releasing adrenaline and other "fight or flight" hormones, keeping you constantly on high alert. Furthermore, during stressful times, cortisol causes your liver to release glucose, or sugar, for quick energy[23].Acute stress is an adaptive, transient state. On the other hand, chronic stress is a persistent state that is linked to maladaptive response, suggesting detrimental impacts on physiological processes[24,25]. Extended periods of stress induce the body to secrete specific hormones or chemicals that signal the ongoing stressful state of the body and impact key organs like the heart, brain, or liver in different ways that may not be beneficial to the patient's health. The body has numerous mechanisms that either separately or together control stress levels,[26,27] including the immunological system, the autonomic nervous system (ANS), and the hypothalamic-pituitary-adrenal axis (HPA-Axis)[28-31]. Because of this connection, the HPA axis is regarded as the mediating system and is used to assess how stress affects disease processes. It is also crucial for immunological response, behaviour, metabolism, and cognition[32]. While the body's cortisol level typically stays elevated throughout certain periods of the day, such as the morning, it is concerning when this state of affairs persists all day. Saliva, blood, urine, and hair samples are among the biological media that can be used to measure cortisol levels[26]. As previously noted, the ANS plays a role in the production and management of both acute and chronic stress. It controls body functions by autonomic responses in reaction to internal (maintaining body homeostasis, including temperature, blood sugar, elimination of excess fluids, obesity, etc.) and external stimuli (environment, vision, smell, touch, etc.) [33,34-37].

1.3Pathogenesis and causes of stress

Stress can be either acute or ongoing. While both can have a variety of negative side effects, prolonged stress can be harmful to one's health in the long run. The primary hormones that are released in reaction to stress are catecholamines and glucocorticoids. In the short term,

these hormones have no negative consequences, but over time, they may disrupt glucose homeostasis. Type II diabetes and insulin resistance can result from this disruption of glucose homeostasis, which can cause chronic hyperglycemia [47].

1.4Effect of stress on cortisol level

The steroid-shaped hormone cortisol has a glucocorticoid impact and is secreted from the suprarenal gland's outer cortex [38,39].It mainly diffuses passively from capillary arteries to cells because of its tiny, fat-soluble nature [39,40] It is found in bodily fluids such urine, sweat, hair, saliva, and cerebrospinal fluid [41]. There is a circadian oscillation rhythm in cortisol. Early in the morning is when blood cortisol levels are highest, and midnight is when they are lowest [39-41]. A minor portion of the cortisol in blood is conveyed as bound to albumin, while the majority is strongly bound to corticosteroid-binding protein (CBG, also known as transcortin). Only 3–10% of the total cortisol in blood is detected as free cortisol [39,40].It has been observed that salivary cortisol concentrations correspond to blood free cortisol levels [39-42]. Salivary cortisol is a crucial parameter in psychoneuroendocrinological monitoring since it can measure free cortisol levels noninvasively, doesn't hurt, and makes it simple for a person to obtain a sample on their own whenever they wish [43]. It was discovered that during stressful situations, cortisol levels increased almost nine times more than during calm times. A few physical reactions to stress include trembling, cramping, myotonia, muscle spasms, and numbness in the fingers and toes. [44-46].

1.5Effect of stress on blood glucose level

Hormones released in reaction to stress may cause blood sugar levels to increase. In a healthy patient, this has adaptive value, but over time, it can result in insulin resistance and diabetes. Furthermore, irregularities in the way these stress hormones are regulated may result from diabetes. Glycogen is depleted and gluconeogenesis is accelerated by glucocorticoids. Because it prevents white adipose tissue and muscles from collecting and utilizing glucose, hyperglycemia is the most common and rapidly apparent adverse consequence [47]. Insulin resistance, visceral fat buildup, and leaner body mass loss can all be brought on by prolonged stress. Glucocorticoids counteract insulin's metabolic effects[48,49].

Insulin stimulates the glucose transporter type 4 (GLUT-4), which is the main regulator of glucose uptake and is found in muscle. The inhibition of GLUT 4 translocation to the cell surface in response to insulin occurs when glucocorticoids are present. This leads to a decrease in the skeletal muscles' capacity to absorb glucose, which raises blood glucose levels [50,51]. In white adipose tissue, glucocorticoids increase lipolysis to generate glycerol, a precursor to gluconeogenesis. This facilitates the build-up of non-esterified fatty acids in muscle cells, which again reduces glucose uptake by disrupting insulin signaling. As a result, the body uses glucose less effectively and becomes hyperglycemic. Additionally, corticosteroids inhibit the pancreatic cells' ability to produce and secrete insulin.[52,53]

1.6Effect of stress on body weight

Gaining weight can result from ongoing stress. Fortunately, there are easy and efficient ways to manage your weight by lowering everyday stressors. Only a thorough medical history and the exclusion of other possible causes of weight gain, such as low thyroid function, can be used to diagnose stress-related weight gain.

1.7Effect of stress on spleen structure

Stress can induce spleen involution, which hinders the host's capacity to generate an immune response[54]. Immune function was significantly altered by hydrocortisone, as evidenced by increased red pulp and decreased white pulp. This phenomenon may be explained by the long-term exposure of T and B cells to high in vivo corticosterone levels desensitizing their glucocorticoid receptors, which inhibits cell proliferation [55]. Long-term stress causes anatomical changes that may modify humoral and cellular immune responses.

1.8Opportunities in anti-stress activity from medicinal plant

Plants are utilized as medicines in herbal remedies. Herbal remedies are being used by people all over the world as an alternative form of medicine to help prevent, treat, or cure diseases. They are also used to relieve symptoms, increase energy, and relax. It is thought

that these remedies have no negative side effects [56, 57]. Many medicinal plants and the active components in them have been studied on various disease models and shown to have anti-disease properties [58-60]. Likewise, a number of plant extracts from herbal medicine, or their purified fractions, have been subjected to molecular testing and have been shown to have anti-stress properties by raising neurotransmitter levels. They have also been employed as immune system enhancers [61]. There have been reports of anxiolytic, antidepressant, neuroprotective, and memory-enhancing properties for bacopamonniera, commonly known as brahmi [62]. Bacopamonniera, also referred to as brahmi, has been shown to have anxiolytic, antidepressant, neuroprotective, and memory-enhancing qualities [63]. Most significantly, applications of Panax ginseng have also been shown to boost immunity and have antiviral properties [64]. Additionally, using Panax ginseng topically has been shown to delay the onset of acute respiratory tract infections [65]. Withaniasomnifera, popularly known as ashwagandha, is another herb that has been shown to have potential for lowering stress and anxiety levels as well as assisting people in overcoming depression and enhancing brain function [66]. In addition, Ocimum sanctum, also known as tulsi, is a different medicinal plant that is well-known for its numerous therapeutic uses, which include antimicrobial, anti-stress, and immune-boosting properties [67,68]. Ocimum sanctum has also been demonstrated to counteract metabolic stress by restoring normal blood pressure, cholesterol, and glucose levels. Its anxiolytic and antidepressant qualities have also been shown to enhance memory and cognitive abilities [67]. Furthermore, Ocimum sanctum has been shown to alleviate central monoaminergic disturbances, antioxidant system disruptions, and may have therapeutic potential for the treatment of early or mild flu [67,68].

1.9Prevention of stress by neutraceuticals or natural plants

Known as "phytochemicals," a diverse range of alkaloids, polyphenols, and terpenoids are found in plants, fruits, and vegetables. The majority of these compounds are in charge of giving fruits and vegetables their beneficial qualities. These foods are vital to a healthy lifestyle because they can help prolong life and treat chronic illnesses. Nutraceuticals are ingredients, or portions of ingredients, in food that have a positive impact on health and can help prevent or treat certain conditions[69]. Folk medicine has employed plant-extract based remedies for centuries. In recent years, research has concentrated on identifying plant-based

bioactive compounds that have the potential to positively impact human physiology. In actuality, natural ingredients are the source of some of the most basic medical interventions. A lot of interest was generated in recent years by demonstrating potential plant-based medications, and this presented a challenge for many researchers [70]. In addition to food, nutraceuticals—food or their extract—can be utilized to prevent oxidative stress by offering additional health benefits. Because of their innate antioxidant qualities, a whole class of nutraceuticals can be incorporated into our regular diets to help avoid oxidative stress. By regulating the consumption of nutraceuticals, oxidative stress can be avoided by maintaining a stable redox state. Nutraceuticals can help control conditions like diabetes, neurodegeneration, cancer, organ inflammation, cardiovascular diseases, and other conditions like that which are brought on by cellular oxidation in addition to oxidative stress management [71]. Although they offer a sustainable and successful preventive strategy, nutraceuticals have a toxic profile that cannot be disregarded. Any deviation from the recommended dosage could be lethal [72]. .. Furthermore, the majority of the purported nutraceuticals have not undergone a thorough safety evaluation because they lack the necessary resources. The pooling of bioactive compounds makes it challenging to analyze the pharmacokinetic characteristics of the nutraceuticals under test [73].

1.10Bio markers for stress

A cortisol test determines whether the amounts of cortisol in your saliva, urine, or blood are normal. The hormone cortisol has an impact on nearly all of your body's tissues and organs. It benefits your body. React to stress (the "stress hormone" cortisol is sometimes referred to as).

The adrenomedullary hormones norepinephrine and adrenaline, salivary alpha-amylase, pro- and anti-inflammatory cytokines, c-reactive protein (CRP), and stress-sensitive cardiovascular measurements are some of the specific biomarkers that will be covered.

1] Cortisol level

2]Blood sugar level

3] Body weight

4] Spleen structure

1.11Objective of the study

General Objective:

To determine the possible therapeutic benefit of extracting the seeds of the jackfruit pl (Artocarpus heterophyllus) by studying its influence on stress modulation in experimental mice.

Specific Objective:

- To determine the phytochemical screening, anti stressed activity of plant extract.
- To find out effects of *Artocarpus heterophyllus* on glucose level and hormone level (cortisol)
- To find the effects on body weight and spleen structure.
- Seeking for a nutraceutical benefits from plant extract of *Artocarpus heterophyllus* which have minimum side effect.

CHAPTER 2

PLANT PROFILE

2.1 Introduction of *Artocarpus heterophyllus*

Scientific Name: *Artocarpusheterophyllus*

Common Name: Jackfruit , Jackfruit seed, Jacknut

English name: Jackfruit seed

2.2 Scientific classification of*Artocarpus heterophyllus*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Urticales

Family: Moraceae

Genus: Artocarpus

Species: Artocarpusheterophyllus [74]

Figure 1 : Jackfruit seed

2.3 Distribution

When Artocarpusheterophyllus reaches maturity, its growth rate slows to approximately 0.5 m/yr (20 in/yr). In its early years, the tree can grow up to 1.5 m/yr (5 ft/yr) in height [75].Since ancient times, jackfruit has been farmed and has spread over many tropical regions, especially in Southeast Asia, where it is currently a major crop in the countries of

India, Myanmar, China, Sri Lanka, Malaysia, Indonesia, Thailand, and the Philippines. A portion of Africa, Brazil, Surinam, the Caribbean, Florida, and Australia are also home to its cultivation. Since European settlers arrived on numerous Pacific islands, it has spread, and in Fiji, home to a sizable Indian-descent population, it is especially significant.

2.4 Active constituents

The chemical components of Artocarpusheterophyllus include numerous flavones, which are colouring agents, as well as morin, dihydromorin, cynomacurin, and artocarpin,artocarpesin, oxydihydroartocarpesin, cycloartinone, artocarpetin, norartocarpetin, isoartocarpin, and cyloartocarpin[76].Upon investigation, the heart wood produces the following results: 6.7% moisture, 38.0% glycosides, 0.7% lipids, 1.7% albumin, and 59.5 % cellulose[77].Along with free sugar (sucrose), fatty acids, ellagic acid, and a few important amino acids including histidine, arginine, cysteine, leucine, lysine, metheonine, theonine, tryptophan, etc., the plant also contains these other nutrients[78].The seeds have constipating and diuretic properties.The wood contains nervine, antidiabetic, sedative, and convulsive properties.[79]

2.5 Pharmacological Uses

Even though the ingredients in it have been the subject of several pharmacological studies, there is still much more that can be discovered and applied therapeutically.

Anti-inflammatory Effect

Large evergreen trees called *Artocarpusheterophyllus* Lam are grown for their fruits all over Southeast Asia. Its roots and leaves have been utilised medicinally [80].Artocarpesin, which is present in the fruits, inhibits the production of prostaglandin E2 (PGE2) and nitric oxide (NO), potentially offering an anti-inflammatory effect [81].

Antioxidant Effect

Antioxidant properties against lipid peroxidation are exhibited by phenylflavones isolated from Artocarpus heterophyllus, such as cycloheterophyllin and artonins A and B.[82]

Immunomodulatory effect

The primary protein found in seeds, jacalin, has been found to be useful in the isolation of human plasma glycoproteins, the study of IgA-nephropathy, the examination of O-linked

glycoproteins, and the identification of tumors.[83]

Antifungal Effect

The lectin that binds to chitin found in the seeds known as jackin prevents Fuseriummoniliforme and Saccharomyces cerevisiae from growing.[84]

Antidiabetic Effect

Patients with diabetes have shown to have improved glucose tolerance when exposed to hot water extracts of Artocarpus heterophyllus leaves.[85]

Antibacterial Effect

Broad spectrum antibacterial activity is exhibited by the crude extract of barks, stems and roots, stem and root heart-wood, leaves, fruits, and seeds combined with methanol.[86]

CHAPTER 3

LITERATURE REVIEW

3.1 PHYTOCHEMICAL, NUTRITIONAL AND ANTIOXIDANT ACTIVITY EVALUATION OF SEEDS OF JACKFRUIT (ARTOCARPOUS HETEROLPHYLLUS LAM.) DEEPIKA GUPTA, SONIA MANN, AVIJIT SOOD AND RAJINDER K. GUPTA* University School of Biotechnology, GGS Indraprastha University, Dwarka, New Delhi 110075, India.

In the current study, we examined the nutritional, phytochemical, and antioxidant properties of jackfruit (Artocarpusheterophyllus Lam.), one of the oldest fruits that is native to India's Western Ghats. Free radical scavenging, metal chelating, ferric reducing, antioxidant power, and reducing power assays were used to assess the antioxidant capabilities. The jackfruit seeds' secondary metabolite content, which included alkaloids, saponins, flavanoids, and phenolics, was assessed.The seeds' nutritional characteristics, such as moisture content, lipid content, carbohydrate content, protein content, ash content, and metal content, were also assessed.Dichloromethane:Methanol (1:1) extract of jackfruit seeds was shown to have a significant polyphenolic content and well-correlated antioxidant activities. The findings showed that jackfruit seeds are a good source of nutritional and antioxidant components and have the potential to be enhanced and turned into nutraceuticals. [87]

3.2 Nutrient and Phytochemical Composition of Jackfruit (Artocarpusheterophyllus) Pulp, Seeds and Leaves *Amadi, Joy A.C1 ., Ihemeje, Austin2 and Afam-Anene, O.C1.

The purpose of the study was to assess the nutritional and phytochemical content of jackfruit pulp, seed, and leaves. A local farm in Obiangwu, NgorOkpala Local Government Area, Imo State, Nigeria, was used to collect jackfruit. Utilising accepted techniques, the compositions of nutrients and phytochemicals were assessed. With the help of statistical product for service solution (SPSS) version 22.0, means and standard deviation were calculated. Calculating and separating the means involved using the Turkey test and one-way analysis of variance (ANOVA). The cutoff for statistical significance was $P< 0.05$. While crude fibre (4.91± 0.06) and ash (2.53± 0.06) were considerably ($p<0.05$) higher in the jackfruit leaves, crude protein (10.09± 0.11), fat (4.29± 0.12), and carbohydrate (7.89 ±0.13) were also significantly greater in the jackfruit seed. The micronutrient composition reveals that the leaves were substantially ($p<0.05$) higher in calcium (0.52±0.01), manganese (12.75 ±0.35) and iron (59.50±0.71) than the pulp was in potassium (0.33± 0.01), vitamin C (2.10± 0.01) and zinc (9.28± 0.11).Jackfruit pulp had the lowest levels of phytic acid, oxalate, alkaloids, tannin, and flavonoid (6.14, 3.69, 7.88, 0.03, and 3.91) according to the phytochemical composition. Jackfruit leaves had higher levels of alkaloid (7.88±0.06), tannin (0.06±0.01), and flavonoid (2.03±0.06), whereas the seeds had higher levels of phytic acid (8.11±0.06) and oxalate (5.53 ±0.13). The results of the study showed that the jackfruit's pulp, seed, and leaves are full of nutrients.The pulp, seed, and leaves of the jackfruit contain phytochemicals that will improve health, particularly in the battle against non-communicable illnesses. [88]

3.3 The Effect of Jackfruit (Artocarpusheterophyllus Lam.) Seed Ethanol Extract on Blood Sugar Levels and Anti-Inflammatory Reduction on Wistar Albino Rats Streptozotocin-Induced Gestational Diabetes

Basaria Manurung1, Hadyanto Lim2, JeksonMartiar Siahaan2, EndyJuli Anto3,Putri C Eyanoer4, Sandeep Poddar5

It was during the second and third trimesters of pregnancy when gestational diabetes mellitus, a condition of carbohydrate tolerance that causes elevated blood sugar levels, was first identified. It is a medical condition that directly affects both the mother's and the fetus's health. This study looked at how jackfruit (Artocarpusheterophyllus Lam.) seed ethanol extract affected inflammation and blood sugar levels in Wistar albino rats with gestational diabetes mellitus that was brought on by streptozotocin. On Wistar albino rats with gestational diabetes mellitus, this study used a laboratory experimental research design with a post-test only control group design. The sample was obtained using a straightforward random sampling technique. The findings revealed that group C, which received streptozotocin 45 mg/kg BW plus metformin 45 mg/kg BW, had the lowest spectrophotometer KGD level, with a p value of 0.003, indicating a significant difference between groups and the lowest Interleukin-6 level. With a p value of 0.511, group C's treatment regimen of 45 mg/kg BW of streptozotocin and 45 mg/kg BW of metformin resulted in no discernible difference between the groups. Jackfruit seed ethanol extract has anti-inflammatory and blood sugar-lowering properties.[89]

3.4 Stress and the Hormonal Regulation of the Immune Response in Mice

Adrenocorticotropic hormone injection or exposure of mice to various forms of acute stress (acceleration, ether anaesthesia, restraint, overcrowding) led to an increase in plasma corticosteroid levels. This was connected to their spleen cells' in vitro immunological reactivity being lower. On the other hand, there was no immunosuppression seen following repeated exposure to ether stress. Before acute stress, cell donors were pretreated with diazepam, which blocked the immunosuppressive effects of confinement but had no effect on the negative effects of ether anaesthesia. Desipramine, on the other hand, had no effect on either kind of stress reaction. By including macrophages and B cells from healthy mice, inactive spleen cell cultures might be brought back to life. No amount of macrophages, B or T cells by themselves, or T cells together with either macrophages or B cells, demonstrated any healing effects. The homing of B and T cells from Cr-labeled lymph node cells from normal nu/+ mice to the spleen and bone marrow was dramatically accelerated by restraint stress. In contrast, stress-induced recipients showed a decreased homing of nu/nu lymph node cells (B cells) to lymph nodes and spleen and an increased proportion of cells in the liver. An immunological responsiveness reduction caused by hypophysectomy in cell donors was eventually reversed by somatotropic hormone (STH) therapy. STH probably speeds up the process of recovering from stress-related immunosuppression. Exogenous STH also counteracted the effects of elevated endogenous corticosterone. [90]

3.5 HEPATO-PROTECTIVE EFFECT OF AQUEOUS EXTRACT OF SEED, LEAF AND FRUIT OF JACKFRUIT (ARTOCARPUS HETEROPHYLLUS LAM.) AGAINST CCL4 INDUCED HEPATOTOXICITY ON SWISS ALBINO MICE.Homen Phukan1,2 *,Laishram I. Singha1 and Pradip Kr. Mitra2 1 (Department of Biotechnology and biochemistry, St. Anthony‟s College, Shillong, Meghalaya, India-793001). 2 (Institutional Biotech Hub, North Lakhimpur College (Autonomous), Lakhimpur-787031, Assam, India).

In Indian home gardens, the jackfruit tree (Artocarpusheterophyllus Lam.) is one of the significant and frequently encountered trees. It is a common substance in traditional medicine and contains secondary metabolites with therapeutic importance. Swiss albino mice were

used to test the effects of this plant's seed, leaf, and fruit aqueous extract on CCl4-induced hepatotoxicity. After receiving CCl4 treatment for one day on animals of either sex, a consistent dose of aqueous Jackfruit seed, leaf, and fruit extract was given orally for 30 days. The serum biochemical parameters were evaluated in order to ascertain the extracts' hepatoprotective efficacy. Animals treated with CCl4 had dramatically altered serum levels of ALP, AST, ALT, bilirubin, and total protein. With the exception of total bilirubin level, these levels returned near to normal levels after ingestion of aqueous extract of Jackfruit seed, leaf, and fruit. According to the current study, the aqueous extract of jackfruit fruit, leaf, and seed has advantageous effects on liver function. This hepatoprotective action is thought to be brought on by high calcium, potassium, and magnesium contents as well as secondary metabolites that react to environmental stimuli. [91]

CHAPTER 4

MATERIALS & METHODS

4.1 Collection of plant sample

The fruit's seed of *Artocarpus heterophyllus* was collected from local area. It was collected in September 2023 at day time. During collection of the fruit's seed any chance of adulteration, hydrolysis, or chemical degradation was avoided. The fruit that were attached to the fruit's seed were removed by using knife.

4.2 Preparation of plant sample

The fruits were collected in fresh condition. Then the seed was separated from the fruit. These were cut into small pieces and make it suitable for grinding purpose and drying. The seed was dried by shed drying for about fifteen days, and finally the pieces were dried in an oven at 40-45oC for 30 minutes to remove the remaining moisture by ensuring the seeds became crispy. The peels are grinded into coarse powder with the help of a grinder and stored in an air tight container and placed in a dark, cool & dry place for further use.

4.3 Extraction

A glass made jar with plastic cover was taken and washed thoroughly the jar was rinsed with

methanol. Then 400 gram of the dried leaves was taken in the jar, and that methanol (1200 ml) was poured into the jar up to 1-inch height above the sample surface as it sufficiently covered the sample surface. The plastic cover with Aluminum foil was used to close the jar properly to resist the entrance of air into the jar. The aforementioned process was performed for 14 days. The jar was shaken on regular basis almost 2 times daily for a better extraction.

4.4 Filtration

After the extraction process the plant extract was filtered with sterilized cotton and filter paper, the cotton and filter paper was both rainsed with the required solvent and fitted in a funnel. The filtrate was collected in a glass container.

4.5 Evaporation

The liquid extract was evaporated by using rotary evaporator. For the evaporation of methanol, the evaporation was carried out at 65-670C and 34-38 RPM (rotation per minute). The evaporated sample was transferred to a 50ml beaker. After collecting the pure extract, it was placed directly under fan by covering the beakers with aluminum foil. To evaporate the remaining solvent present in the extract, and finally hair dryer (cold) was used to remove some portion of water in the remaining extract.

4.6 Chemical group test

Testing of different chemical groups which are present in extract that represents the preliminary phytochemical studies. The chemical group test, which are performed as follows:

Tests procedure for identifying different chemical groups

Detection of Alkaloids

Mayer's test

2ml solution of the extract and 1% 5 ml of hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent (Potassium Mercuric Iodide) was added. Formation of Yellow color or creamy white precipitate indicates the presence of alkaloids.

Detection of Flavonoids

Few drops of concentrated hydrochloric acid were added into 1ml of crude extract. Immediate formation of red color has shown the presence of flavonoids.

Detection of Saponins

1ml of extract solution was diluted with 20 ml distilled water. Then shaken vigorously for 15

minutes which develops clear foam and it indicates the presence of saponin.

Detection of Carbohydrate

2 ml of extract of the plant material was taken in a test tube. 2-3 drops of Molisch's reagent was added to the test tube, and 2ml of concentrate H_2SO_4 was added . A red color or purple ring show the presence of carbohydrates.

Detection of Glycosides

(i)A small amount of an extract of the fresh or dried plant material was taken in 1ml of water. Then, a few drops of aqueous sodium hydroxide were added. A yellow color was considered as an indication for the presence of glycosides.

(ii) 5ml extract added on 2ml glacial acetic acid as well as 1-2 drops of FeCl solution and 1ml concentrated H2SO4 added, appearance of a reddish brown color show the presence of glycosides

(iii) Another portion of the extract was dissolved in water and alcoholic and boiled with few drops of dilute sulfuric acid, neutralized with sodium hydroxide solution as an indication for the presence of glycosides

Tests for Tanin

5ml extract and 2-4 drops of Ferric chloride solution added on a test tube, greenish black precipitate show the presence of tannin

Tests for Phytosterol

2ml extract added on a test tube then 1ml chloroform and 1ml concentrated sulfuric acid added on this test tube ,golden red or golden yellow color indicates the presence of phytosterols .

4.7 Experimental Design

For this investigation, Swiss albino mice were recruited from the Jahangirnagar University Lab. Female albino mice, five months old and weighing between 26 and 30 g, were utilized in the experiments. For 5 days previous to the start of the experiment, the rats were kept in colony cages in the department's temperature-controlled animal room (25 – 30 °C). The bedding was changed every day to guarantee cleanliness and hygiene.

For stress induction hydrocortisone was administered.

For the study the mice were divided into four groups and each group consist of n=3 mice. The four groups were

- G-1 Control
- G-2 Stress
- G-3 Stress + Drug
- G-4 Stress+Extract

4.8 Medication & Diet

Table 1: Medication & sample extract performed throughout the study

Group	Medication & Sample	Duration
G-1 Control	Normal water	7 days
G-2 Stress	Hydrocortisone	7 days
G-3 Stress + Drug	Hydrocortisone+Clonazepam	7 days
G-4 Stress+Extract	Hydrocortisone + extract	7 days

4.9 Blood Collection

The mice were slaughtered and blood samples taken after 7 days of successful administration of the experimental drugs and samples. A 2–3 ml blood sample was obtained using the heart puncture method. After centrifuging the blood, the serum was collected and stored for future research.

4.10 Biochemical Test

Two biochemical tests were performed as part of the inquiry. Cortisol is the first, while the blood glucose level is the second.

4.11 Morphological Study

The mice were slaughtered when the research period finished, and their average body weights were reported. After that, the key organs (spleen) were removed and physical appearance changes such size were compared to the control group. The spleen was dissected to look for any symptoms of stress.

CHAPTER 5

RESULTS& DISCUSSION

5.1 Different chemical group test result

Table 2: Phytochemical screening

Phytochemical constituents	Result
Alkaloid	-
Flavonoids	+
Carbohydrates	+
Tanins	-
Steroids	+
Diterpenes	+
Phytosterol	+
Saponin	-
Glycoside	+
Gum	-

Discussion: [+] Indicates **presence**

[-] Indicates **absence**

5.2. Effects on body weight

Table 3: Effects on body weight throughout the study

Group Name	Medication	Initial weight (gm)	Final weight (gm)	Weight variations (gm)
G-1 Control	Normal water	28 ±1	28.66 ± 0.33	0.66gm (Slight increased)
G-2 Stress	Hydrocortisone (2.047mg/kg)	26.33±1.20	26.66±1.20	0.33gm (Slight increased)
G-3Stress+Drug (standard)	Hydrocortisone (2.047mg/kg)+Clonazepam	26.66± 1.20	26.66± 0.88	No change

	(0.103mg/kg)			
G-4 Stress+Extract (sample)	Hydrocortisone (2.047mg/kg)+AH(0.5g/ kg)	27 ± 0.57	27.66±0.33	0.66gm (Slight increased)

Data were expressed as mean ± SEM (Standard Error Mean) where n= 3 for single group.

The starting and ending weights of the experimental animals, as well as their changes throughout time, were seen in table 10. The control group's weight slightly change , and the only weight gain after the study was 0.66 grams. There was some tiny change in weight in the stress group with a gain of 0.33 gm from the initial weight. On the other hand, it was strikingly discovered that the sample group's weight was 0.66 grams increased and that there was no weight variance in the standard group.

As a result of following a regular, healthy diet for the duration of the trial, there were no significant changes in the weight variation of the any group. Because the sample treatment had a significant quantity of fiber, . According to reports, increasing fiber in the diet can help manage obesity [171].

5.3 Cortisol test Result:

Table 4: Effects of sample (Jackfruit seed) on cortisol a biomarker for stress function.

Group Name	**Medication**	**Cortisol level (µg/ dl)**
G-1 Control	Normal water	3.09± 0.49
G-2 Stress	Hydrocortisone (2.047mg/kg)	4.17±0.19
G-3 3Stress+Drug (standard)	Hydrocortisone (2.047mg/kg)+Clonazepam(0.103 mg/kg)	3.17 ±0.18
G-4 Stress+Extract	Hydrocortisone	3.45±0.14

(sample)	(2.047mg/kg)+AH(0.5g/ kg)	

Data were expressed as mean ± SEM (Standard Error Mean) where n= 3 for single group.

Serum cortisol levels in the normal control,stress control, standard and sample groups, as well as the acceptable limits for mice, are shown in Table 4. Since the normal group value is 3.09**(**µg/ dl), the cortisol level of both standard and sample groups are normal,with values of 3.17 **(µg/ dl)** and 3.45(µg/ dl), respectively.The stress group increased as compared to the control group and the value is 4.17(µg/ dl), , according to analysis of the cortisol test result.As can be understood from the above result that this sample group are able to decrease serum cortisol level.

Table 5: Effects of sample on blood glucose level

Group Name	Medication	Initial blood glucose(mmol/L)	Final blood glucose(mmol/L
G-1 Control	Normal water	5.86±0.49	4.46±0.44
G-2 Stress	Hydrocortisone (2.047mg/kg)	7.43±0.89	10.23±1.08
G-3 Stress+Drug (standard)	Hydrocortisone (2.047mg/kg)+Clonazepam (0.103mg/kg	8.3± 0.26	6.86±1.01
G-4 Stress+Extract (sample)	Hydrocortisone (2.047mg/kg)+AH(0.5g/kg)	9.16± 0.38	11.33±0.48

Data were expressed as mean ± SEM (Standard Error Mean) where n= 3 for single group.

In the sample group, total blood sugar levels were 11.33(mmol/L) compared to10.23 (mmol/dl) in the stress group. As a consequence, it is assumed that sample treatment caused

the 1.13 mmol/dl level to raise. The glucose levels increased in overall groups by compared to the control, an outstanding results was marked out in standard group where the standard group values for glucose was 8.3 (mmol/L)in initial and 6.86(mmol/L) .And there was a noticeable difference between the two results.This is a great opportunity to look into the sample extract even more and a fantastic chance for additional investigation on the sample.

5.4 Morphological Investigation of Spleen

In morphological study of spleen ,it was found that the sample ,control & standard group spleen size was smaller than the stress group which indicates medicinal plants and standard drugs are capable in controlling stress.

Table 6: Comparison of spleen size among experimental animals

Group Name	**Medication**	**Spleen size (cm)**
G-1 Control	Normal water	1.6± 0.11
G-2 Stress	Hydrocortisone (2.047mg/kg)	1.8 ±0.05
G-3 Stress+Drug (standard)	Hydrocortisone (2.047mg/kg)+Clonazepam (0.103mg/kg)	1.5 ±0.11
G-4 Stress+Extract (sample)	Hydrocortisone (2.047mg/kg)+AH(0.5g/kg)	1.6±0.05

Data were expressed as mean ± SEM (Standard Error Mean) where n= 3 for single group.

From the table we can see the mice spleen in the control, sample and standard group were 1.6±0.11 cm,1.6±0.05 cm and 1.5 ±0.11cm where stress group 1.8 ±0.05. The sample group and standard had a decrease in spleen size as result of stress induction. Where as the spleen size of the experimental and comparion group was considerable higher.

CHAPTER 6

CONCLUSION

6.1 Conclusion

The *Artocarpus heterophyllus* seed extract is the subject of the thesis work. The thesis work that is being presented here addresses various studies on. Research was done in the fields of pharmacology and phytochemistry.The assessment of phytochemicals revealed the absence of alkaloid, tanins, saponin, gum and the presence of flavonoids, carbohydrates, steroids ,diterpenes, phytosterol, glycoside.The major goal of this study has been to determine how jackfruit seed (*Artocarpus heterophyllus*) affected glucose levels and hormone levels (corticosterone), as well as how hydrocortisone administration affected body weight and spleen structure.In addition to being tested for phytochemical screening and anti-stress activity, the jackfruit seed sample extract was also examined for potential nutraceutical benefits from the plant extract that had the fewest negative effects.The results of the experiments in this study demonstrated that the methanol extract of *Artocarpus heterophyllus* seed contained organic compounds that may be largely responsible for the pharmacological activity.The present evaluation also showed mild antistress activity.This study was conducted using crude seed extract of *Artocarpus heterophyllus*.Additionally, more extensive research in that area should provide more details regarding these allegations.

CHAPTER 7

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