THE EFFECT OF ETHANOL EXTRACT OF Solatium nigrum LEAVES ON THE CONCENTRATION OF MALONDIALDEHYDE (MDA) AFTER HISTAMINE CHALLENGE IN OVALBUMIN SENSITIZED GUINEA PIGS

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CERTIFICATION

This is to certify that this work was carried out by OFEINMU ABRAHAM EIHIZOJIE, MAT NO. LSC1705195 of the department of Biochemistry, faculty of life sciences, University of Benin, Benin city, as part of requirement for the award of Bachelor of Science degree (B.Sc. Hons.) in Biochemistry.

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DEDICATION

This project research is dedicated to God almighty for the wisdom, knowledge, and understanding he imparted into me through Jesus Christ for the successful completion of this research. And I also want to thank my parents Mr. and Mrs. Ofeinmu, for their innumerable support, all through my time in the school.

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God bless you all.

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ABSTRACT

In this study, the effect of ethanol extract of Solarium nigrum (Sn) leaf on the concentration of malondialdehyde (MDA) after histamine challenge in ovalbumin-sensitized guinea pigs was investigated. Three weeks old, male guinea pigs (25 of them) were divided into five groups consisting of five pigs each were used. All animals were allowed access to standard animal feed and water. In addition animals were pre-sensitized with ovalbumin, and then exposed to 2% histamine aerosol after an overnight fast. The pre convulsion time was recorded. Animals in groups 2, 3, 4, were administered ethanol extract of Solanum nigrum leaf at the respective doses of 50, 100 and 200mg/kg body weight while animals in group 5. received 25mg/kgbw of the reference drug aminophylline. Animals were again subjected to histamine aerosol after drug administration and the pre-convulsion was again recorded. All animals were sacrificed three days after the last challenge and their lungs were excised. Result obtained indicates that sensitization with ovalbumin and exposure to histamine aerosol caused the increase in the lipid peroxidation in bronchial alveolar tissue, lung and plasma cells respectively. Treatment with ethanol extract of Solanum nigrum (Sn) leaf showed a decrease in the concentration of malondialdehyde (MDA) when compared to that of the control.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0 INTRODUCTION

Asthma is a condition in which there is variable breathlessness due to widespread narrowing of the peripheral airway which varies in severity over short period of time, either spontaneously or with treatment. Attacks of asthma usually respond to simple bronchodilators drugs. However, in this era of traditional medicine, herbs are being sort for that can cure or at least effectively manage asthma. One of such plants, *Solanum nigrum L*, is a common weed of wasteland and edges of cultivated land in most parts of the world and has been used mainly as a topical ointment in the treatment of injuries (Happer, 1965), yaws (British Guideline 2009), and lumbago (Jindal, 2011).There have been several claims by traditional drug users that *Solanium nigrum L* has anti-asthmatic properties, the purpose of this study is to determine the authenticity of these claims.

Neurotransmission, immunomodulation (enhanced eosinophil and neutrophil chemotaxis, production of prostaglandins and thromboxane B, suppressed synthesis of lymphokines, etc.), haemopoiesis, wound healing, intestinal ischaemia, day-night rhythm, the control of histamine- and polyamine-induced cell proliferation, and angiogenesis in tumor models are all processes in which histamine is involved (Hungerford JM., 2010) (Luss LV., 2014). 3,4 Histamine's interaction with several cell type of membrane receptors mediates its pleiotropic effects. Histamine receptor 1 (H1R), histamine receptor 2 (H2R), histamine receptor 3 (H3R), and histamine receptor 4 (H4R) are the four subtypes currently known. These receptors are all a part of a group of receptors that work in tandem with G-proteins. They are heptahelical transmembrane molecules that transduce extracellular signals through the intracellular second messenger system and G-protein (Nakamura T., *et al*. 2000).

Histamine is created when the enzyme l-histidine decarboxylase, which contains pyridoxal phosphate (vitamin B6), decarboxylates the amino acid histidine.

Since histamine is found in numerous bodily tissues, its name is derived from the Greek histos, which means tissue. In 1927, Best *et al*. isolated it from liver and lung tissues. Gastric enterochromaffin cells, histaminergic neurons, mast cells, and basophils are the body's traditional

sources of histamine. These cells store histamine in intracellular vesicles, from which it is released in response to stimulation. The release of histamine and mast cell degranulation are known to be caused by a specific antigen bonding to the FcRI receptor; this bonding can be prevented by the flavonoid luteolin, which has antioxidant effects (Kritas SK. *et al.,* 2013)
Additionally, activation of mast cell can also occur in non-immune stimuli such as neuropeptides (substance P), components of complement systems (e.g., C3a and C5a), cytokines (IL-1, IL-3, IL-8, and GM-CSF), platelet activating factor (PAF), hyperosmolarity, lipoproteins, adenosine, superoxidases, and hypoxia can cause the activation of c mast cell. Histamine release can be caused by a variety of chemical and physical conditions, including high temperatures, trauma, vibrations, alcohol, as well as some specific foods and medications (Maintz L., *et al.,* 2007). The activation of mast cell plays an important role in the pathophysiology of many diseases such as allergies also autoimmune such as rheumatoid arthritis (Kritas SK., *et al.,* 2013). Also, the ability of de novo synthesis of histamine is also present in other cell types, such as platelets, dendritic cells, neutrophils, monocytes macrophages, and lymphocytes.

1.1 LITERATURE REVIEW

1.1.1 Solanum *nigrum*

Solanum nigrum Linn. (Sn) commonly known as Black Nightshade is a dicot weed in the Solanaceae family. African paediatric used this plant for several ailments which are the cause of infant mortality especially feverish convulsion. *S. nigrum* is an annual branched herb of up to 90 cm high, with dull dark green leaves, juicy, lanceolate, or ovate, and toothless to slightly toothed on the margins. The flowers of this plant are small and white and five widely spread petals with a short pedicellate. Fruits are small, black when ripe (Cooper and Johnson, 1984). *S.nigrum* is found mainly around, old fields, ditches, waste land, roadsides, and cultivated land. It is a common plant found in most parts of Europe and the African continent. *S.nigrum* is a popular plant due to its toxic content of Solanine, it contains glycoalkaloid in which the highest concentrations in the

unripened berries (Cooper and Johnson, 1984). Although it is considered a rich source of one of the most popular plant poisons, through pharmacological prospects it has proven also to be a reservoir of phytochemicals (Lee and Lim, 2006).

1.1.2 Taxonomical classification

Kingdom: Plantae - Plants

Subkingdom: Tracheobionta - Vascular plants

Superdivision: Spermatophyta - Seed plants

Division: Magnoliophyta - Flowering plants

Class: Magnoliopsida - Dicotyledons

Subclass: Asteridae Order: Solanales

Genus: Solanum - nightshade

Species: SolanumNigrum L. - black nightshade

1.1.3 Morphology

S. nigrum L commonly known as Black nightshade is a dicot weed in the *Solanaceae* family. It is an annual herbaceous plant with height of 30-100 cm, pubescent with simple hairs. The stems are often angular, pubescent-pubescent. The opposite leaves, with whole limb, oval and diamond shape are slightly cogged, the bases are cuneate, 4-10 and 3-7 cm wide, pubescent, entire or coarsely dentate, the apex is obtuse. Inflorescences are extra-axillary umbels, the calyx cup-shaped, the corolla is white, 8-10 mm long, the lobes ovateoblong, pubescent abaxially, ciliate spreading. Filaments are 1-1.5 mm long, anthers oblong, 2.5-3.5 mm. Fruiting 2 pedicels are strongly deflexed. The fruiting calyx is applied to the berry. The flowers are sometimes white veined with

purple, with a flat corolla; they are grouped by 3 or 5 along the stem. The fruits are dull black, globose, 8-10 mm in diameter. The fruits are spherical berry 8-10 mm in diameter, green and greenish yellow to black laying maturity **(**Fig 1.2D**)** the fruits are toxic. Their alkaloid content varies depending on the location of the plant where it grows and depending on the season (Lin *et al.,* 2008).

1.1.4 Distribution and habitat

S. nigrum is widely distributed in various habitats throughout the world, from tropical to temperate regions and from sea level to altitudes exceeding 3500 m (Edmonds and Glidewell, 1977). It is a rather common species in wet woods, wasteland, near river, old field, ditches, roadside and cultivated land. Their ability to flower while still young and their prolific seed production all contribute to the success of these species as widespread weeds making it possible to tolerate wide habitat types. (Henderson, 1974). They are generally found in disturbed habitats, such as roadsides, often on arable land especially the edges of cultivated fields and plantations, in hedgerows, quaysides and, on railway cuttings, rubbish tips, in areas around buildings and houses, under trees, on forest and grassland margins, as garden weeds, on shingle beaches, riverbanks and in gullies. The species mainly colonize moist environments, only occurring in areas of low rainfall when the land is subject to irrigation. This plant grows well in fertile soils, especially those rich in phosphorus or nitrogen.

Fig. 1.1.4.. image of the leaves, flowers, and fruits of *Solanum nigrum*

1.1.5 Chemical composition of *S. nigrum*

S. nigrum is so rich on secondary metabolites such as steroid alkaloids, total alkaloids, and steroid saponins glycoprotein, which showed anti-tumor activity. It also contains, flavonoids, tannins, saponins, glycosides, proteins, carbohydrates, coumarins and phytosterol (Hussain and Pople , 1992). Immature berries of *S. nigrum* contents has large solasodine but this decreases the fruit ripening (Nadkarni 1976). Recently studies showed four new steroidal glycosides alkaloids solasonine, solamargine, α and b solanigrinechez isolated by berries of *S. nigrum*. Chemical analysis of the methanol extract of root and stem shows a steroidal genin saturated identified as tigogenin (glycoside and two spirosestanol furostanol glycosides) by mixed melting point and IR spectroscopy (Ravi *et al.,* 2009). Among the new compounds recently experienced degalactotigonin and solanigrosidesCH two steroidal saponins (called *Nigrum*nins I and II), two new disaccharides (BDthevetopyranosylethyl-(1-4)-BD-oleandropyranoside and BDthevetopyranosyl ethyl-(1 -4)-a-Doleandropyranoside) identified by spectroscopic methods. *S.*

nigrum seeds are source of linoleic acid due to their high fat content (Ravi *et al.,* 2009) and contain solanine, protein, palmitic acid oleic acid, and stearic acid and sitosterol (Ghani 2003). The leaves are rich source of riboflavin. They also contain vitamin and nicotinic acid, citric acid, betacarotene, protein, lipid, , solasonine, steroid alkaloids glycol and solamargine acid. Fruits contain saponins and alkaloids steroidal glycol, solamargine, solanine, solasonine a-b, and solanigrine aglycone, solasodine, Genin steroidal trigogenin**.** All parts and immature green contain steroidal glycosides, as glycoalkaloids. In the genus *Solanum* are important both commercially and ecologically. They are widely considered defensive allelochemicals of plants against predators and pathogens. In economic terms, they are used as raw material for the industrial in place of steroid sapogenin diosgenin production of corticosteroids. The main steroid alkaloids are Solanine and solasonine (Schreiber 1958; Doepke *et al.,*1987; Yoshida *et al.,* 1987).

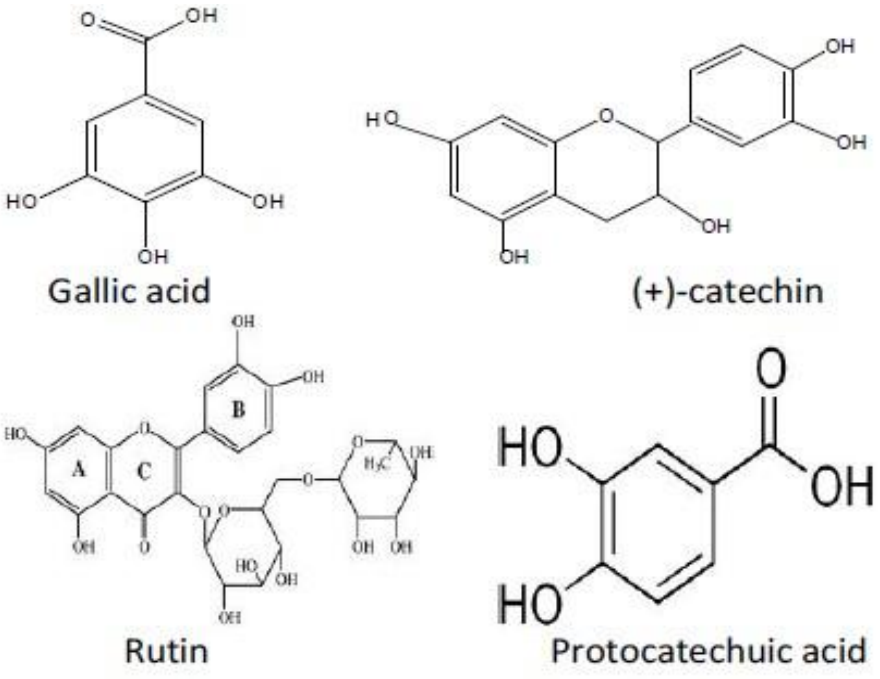


Fig.1.1.5. Chemical constituents of Solanum *nigrum*
(Chauhan *et al.* 2012)

Table 1: Phytochemical Constituents of Solanum *nigrum*

SN. No	Phytochemical	Ethanolic extract	Aqueous Extract	Petroleum Ether Extract
1	Alkaloids	+	+	+
2	Terpenoids	-	-	-
3	Saponins	-	-	-
4	Tannins	+	+	+
5	Flavonoids	+	+	+
6	Phlobatannins	+	-	-
7	Steroid	+	+	+
8	Phenols	-	-	-

(Rjathi *et al.,* 2015)

Table 2: Results of the active substances in the various members of the Solanum *nigrum* plant

Active Substances	Leaves	Twigs	Flowers	Fruits	Roots
Alkaloids	+ + +	+ +	+ +	+ + +	+
Saponins	+ + +	+	+	+ +	+
Tannins	+ +	+	+	+ + +	-
Glycosides	+ + +	+ +	+	+ +	-
Coumarins	+ +	+ +	-	+ +	-
Terpenoids	-	+	-	+ +	+
Flavonoids	+ + +	+ +	+	+ +	-
Volatile oils	-	-	-	-	-

(Djaafar and Ridha, 2014)

(-): Indicates to the absence of an active substance

(+): Indicates the presence of phytochemicals in small quantity

(++): Indicates the presence of phytochemicals in medium quantity

(+++): Indicates the presence of phytochemicals in large quantity

1.1.6 Biological and Pharmacological Activity

The antioxidant activity: Researchers have been succeeding in the isolation of a glycoprotein (150 KDa) from ethanolic extract of fruit of *S. nigrum*. This glycoprotein has scavenging activity against OH, O. radical, DPPH. This glycoprotein has a nonspecific radical scavenging activity in the DPPH assay, which was similar to that of ascorbic acid. So, in the deoxyribose assay for the OH radical, OH radical deduced from deoxyribose degradation is highly removed by the *S. nigrum* glycoprotein. However, it was concluded that *S. nigrum* glycoprotein function as natural antioxidant

(Kung *et al.,* 2004). Also, the protective effects of lunasin against oxidative DNA damage has helped to confirm the antioxidant activity of *S. nigrum.* However, this lunasin peptide compound has been purified from *S. nigrum*, it did not scavenge generated hydroxyl radical, although the lunasin blocked the generation of hydroxyl radical by chelating Fe^{2+} ion (Jin and Ben 2010).

Cardio-protective activity: An *in vitro* total injury ischemia-reperfusion performed using doses of 2.5 and 5.0 mg/kg for 6 days per week for 30 days was used to evaluate the activity of the methanol extract of the fruit. The indication of the results was that the extract exhibited significant ($P<0.001$) of the cardioprotective activity against the ischemia-reperfusion. The activity took place independently of the dose. It was concluded that the methanol extract of berries *S.nigrum* contains a cardio-protective activity (Bhatia and Maiti 2011).

Analgesic activity: Ethanol extracts of *S. nigrum* was evaluated for analgesic activity. The analgesic activity of the extract was evaluated for its central and peripheral pharmacological action

using the hot plate Eddy and acetic acid-induced writhing respectively. The study was carried out using doses of 100, 250 and 500 mg/kg orally, in which the extract shows a positive result of the analgesic activity (Bhatia and Maiti 2011).

The anti-inflammatory activity: Inflammation is a disorder caused by the release of leukocytes and various other complex me33diator such as, leukotrienes, prostaglandins, bradykinin, platelet activating factor, histamines, and IL-1 from tissues and migrating cells.Various drugs and extracts derived from grapes, turmeric, mint, grapes, turmeric, clove, eucalyptus, lavender, and many more have been used to improve inflammation.

In the traditional Indian medicinal system *S. nigrum* is used to treat inflammation, mastitis, and edema. The most widely used method to study anti-inflammatory effects in animals is by inducing local edema in rat paw by injecting an irritant agent such as carrageenan. Were methanol extract of *S. nigrum* showed good dose-dependent anti-inflammatory effect on induced edema in the rat model isolated the mixtures responsible for the anti-inflammatory activity from the ethanolic extract of the *S.nigrum*. Leukotrienes as LTC4 are the lipid mediators that are found in increased concentration in inflammatory reactions. Various inflammatory diseases such as asthma and atopic rhinitis are treated with anti-leukotrienes. (E)-ethyl acetate, one component isolated from *Solanum nigrum*, holds maximum inhibition for leukotrienes and thus could be considered as a potential anti-inflammatory therapeutic compound.

Anti-inflammatory activity in experimental animal models of methanolic of whole plants of *S. nigrum* extract was investigated. The MeOH extract at a dose of 100 mg/kg body weight and 200 mg/kg body weight have shown a significant anti-inflammatory dose-dependent activity. The standard drugs were indomethacin (10 mg/kg) and cyproheptadine (8 mg/kg) (Arunachalam and Subramanian 2009). Ethanol extracts of *S. nigrum* were evaluated for anti-inflammatory activity

using Carrageenan induced rat paw edema. The study was carried out using doses of 100, 250 and 500 mg/kg orally. The anti-inflammatory activity at a dose of 500 mg/kg ($P<0.01$) as compared to the standard drug diclofenac sodium (50 mg/kg) (Kaushik and Jogpal 2009). The effect of methanol extracts of *S. nigrum* fruits were studied on carrageenan induced paw edema. This extract has a way of decreasing the edema induced in the hind paw with a dose (375 mg/kg body weight) showed significant anti-inflammatory (Ghani 2003).

The anti-epileptic activity: Aqueous extract of the leaves of *S. nigrum* was evaluated for antiepileptic activity in mice, chicks, and rats by extract administration by intraperitoneal route. Ata time of 30 min pretreatment and graded doses, animals were challenged with different types of proconvulsant. The result indicates that the aqueous extract of leaves acted significantly ($P<0.05$), dose-dependent protection against electrically induced seizure in chicks and rats, pentylenetetrazol induced seizure in mice and rats and seizure picrotoxin induced in mice and rats. The anti-seizure property of the extract was potentiated by amphetamine. The anticonvulsant potential of herb *S. nigrum* has been revealed with the aqueous extracts with solasodine and diosgenin compounds could be used for treatment of Epilepsy (Noel *et al.,* 2008).

Antibacterial activity: The crude extracts of seeds, leaves, and roots of *S. nigrum* showed a potent antifungal and antibacterial activity (Avat and Mohammad 2013). The methanol extracts of the leaves and seeds of *S.nigrum* show potent activity against all microorganisms namely, *Pseudomonas aeruginosa, Citrobacter, Shigella flexenari*, Staphylococcus aureus*, Escherichia coli, and Yersinia aldovae;Cereviciae Saccharomyces, Aspergillus parasiticus, Trichophytonrubrum, Macrophomina, Fusarium solani and Candida albicans* (Britto *et al.,* 2011). Although, the ethanol extract of the root showed as well antifungal activity against *A. brassicicola* (Muto *et al.,* 2010). The ethanol and ethyl acetate extracts of methanol leaves, the seeds and the

roots were analyzed for their antifungal activity against fungal strains such as *Aspergillus Niger, Penicillium notatum*, and *Trichodermaviridae Fuserium oxisporium* or diameter inhibition was compared to the standard antibiotics. Only extracts from seeds that have antifungal activity (Sridhar *et al.,* 2011).

1.1.7 TOXICITY.

The toxicity of Solanum nigrum varies widely depending on the variety, and

noisonous plant experts advise to avoid eating the berries unless they are a known edible strain (Turner, et al 1971).Toxin level may also be affected by the plant's growing conditions (Edmonds, 1961). All parts of the plant can be poisonous, containing toxic glycolalkaloids at 0.524% (dry weight), Including Solamargine, Solasonines and Solanine (Mohy-ud-dint A, et al 2010.).

The toxins are most concentrated in the unripe green berries, Hartman (Hartman, et al 1983) but also occur in ripe berries. Solanine levels in Solanium nigrum can be extremely toxic and potentially fatal (Hartman, et al 1983) poisoning symptoms are typically delayed for 6 to 12 hours after ingestion. (Schep, et al 2009). initial symptoms of toxicity include fever,sweating vomiting abdominal pain, diarrhea, confusion and drowsiness.(Hartman,et al 1983). Death from ingesting plant parts results from cardiaarrhythmias and respiratory failure (Hartman, et al 1983). Children have died after eating unripe berries and consumption has caused livestock fatalities (North, et al 1977). livestock have also been poisoned from nitrate toxicity by grazing the leaves of Solaniumn nigrum.

1.1.8 The Immune Response

A basic characteristic of asthma associated with allergic sensitization is the ability of the airway to recognize common environmental allergens and to generate a Th2 cytokine in response to them.

Allergen sensitization is based upon the uptake and processing of inhaled allergens by dendritic cells situated in the airway epithelium and submucosa, which then extend their processes to the airway surface (von Garnier *et al.,* 2005; Hammad and Lambrecht 2006). The binding of IgE to high infinity receptors on dendritic cells that facilitate allergen internalization enhances allergen (Kitamura *et al.,* 2007). Once inside the dendritic cell, processing of allergens by cathepsin S and the subsequent selection of peptides loaded onto and presented by HLA molecules (MHC class II) is basis to the ability of these cells to serve as antigen presenting cells to T lymphocytes (Riese and Chapman 2000). Immediately the dendritic cell has engaged allergen, it receives signals to migrate to local lymphoid collections where the antigen is presented. Its specific chemokine receptors, including CCR7 and its ligands CCL21 and CCL19 (and to a lesser extent CXCR4 and its ligand CXCL12), are involved in this chemotactic migration to enable contact with naive T cells (Humrich *et al.,* 2006; Pease and Williams 2006). Presentation of a selected antigen peptide to the T-cell receptor initiates sensitization and the subsequent immune response to the specific allergen (Smit and Lukacs 2006). The nature of this immune response is dependent upon whether engagement of selective costimulatory molecules occurs in parallel. For efficient antigen-dependent the activationT-cell engagement of either CD80 (B7.1) CD86 (B7.2) on the dendritic cells with CD28 on T cells leads to sensitization, while the lack of, or inefficient, engagement of these costimulatory molecules may lead to an abnormal immune response to the antigen (Larche *et al.,* 1998; van Rijt *et al.,* 2004). Also, an alternative method of preventing sensitization and rendering T cells anergic is engagement of a second costimulatory molecule, cytotoxic T-lymphocyte antigen (CTLA)4, which has a higher affinity than either CD80 or CD86 for CD28 and can therefore prevent CD80/CD86 costimulation (Jaffar *et al.,* 1999a, b). In more severe asthma the relative importance of CD28 signaling in supporting the inflammatory response is reduced (Lordan *et al.,* 2001). The capacity of dendritic cells to generate interleukin (IL)-12

determines the balance between Th1 and Th2 responses, IL-12 polarizing T-cell differentiation in favor of a Th1 response (Kuipers *et al.,* 2004). However, while IL-12 can counteract Th2 sensitization, it is also able to contribute to maximal expression of allergic airway disease post sensitization (Meyts *et al.,* 2006). Once sensitized, T cells not only migrate back to the airways to the site of antigen presentation under the influence of the chemokines CCL7, CCL11, CCL13, CCL, CCL22, CCL24 and CCL26 (which interact with their reciprocal receptors including CCR3, CCR4, CCR5, CCR6, CCR7 and CCR8) (Garcia *et al.,* 2005; Kallinich *et al.,* 2005), but these cells also become potent producers of a range of cytokines, namely IL-3, IL-4, IL-5, IL-6, IL-9, IL-13 and granulocyte–macrophage colony stimulating factor (GM-CSF) (Kay 2006; Ryu *et al.,* 2006). IL1b produced by macrophages, monocytes, dendritic cells, and smooth muscle and epithelial cells in large amounts (Schmitz *et al.,* 2003; Dragon *et al.,* 2006) and IL-2 produced by T cells further enhance antigen induced T-cell proliferation and maturation (Anderson 2002). There is now persuasive evidence that at least in mild to moderate asthma Th2-type cells dominate the T-cell repertoire in the airways (Anderson 2002). Through cytokine production, they have the capacity to recruit secondary effector cells such as macrophages, basophils and eosinophils into the inflammatory zone where these cells become primed and subsequently activated for mediator secretion (Fig. 1.2A). Overall, it is the Th2-type T cell bearing the CCR4 chemokine receptor that is the cell which dominates the allergic immune response and may be the cell most probably responsible for contributing to the ongoing chronic inflammatory response.

Mast Cells

The mast cell has long been associated with histamine challenge in cases like asthma. The early asthmatic reaction following inhaled allergen provocation is mast-cell dependent and drugs such as sodium cromoglycate and nedocromil sodium are believed to mediate their effects by inhibiting

mast cell mediator secretion (Holgate 1996). For many years it was thought that the mast cells present in the airway epithelium and submucosa were fundamental to the contribution that these cells make to asthma (Shahana *et al.,* 2005), but recent studies have indicated that mast cells deeper in the airway wall are also important. While undoubtedly mucosal-type mast cells (tryptase positive, chymase negative) under the control of T lymphocytes (specifically IL-3, IL-4 and IL-9) are highly responsive to inhaled allergens (and possibly other stimuli such as hypertonicity) in causing broncho-constriction, there has been recent interest in mast cells present deeper in the airway wall and in the more peripheral airways as being more fundamental to some of the chronic inflammatory responses in asthma (Bradding *et al.,* 2006).The airway smooth muscle is partly dependent on mast cells for its survival and enhanced contractility, whereas mast cells are dependent on smooth muscle factors for their survival and activation. On activation, irrespective of their subtype, mast cells release preformed granule-associated mediators such as histamine, tryptase and other proteases, heparin, and some cytokines, as well as newly formed eicosanoids that include PGD2, thromboxane (TX)A2, and the cysteinyl leukotreines (LTC4 and LTD4) (Bradding *et al.,* 2006). These mediators are potent smooth muscle contractile agents and increase microvascular permeability. Both LTD4 and PGD2 interact with cell surface receptors on eosinophils, macrophages, basophils and mast cells where they serve as chemo attractant as well as priming agents (Ogawa and Calhoun 2006). Activation of mast cells, particularly through the high affinity IgE receptor (FCeRI), leads to the release of certain cytokines that are packaged within mast cell granules, including TNF-α, IL-4 and IL-5 (Bradding *et al.,* 1995; Wilson *et al.,* 2000), but also induces transcription of these and other cytokines and chemokines that are then secreted over a period of up to 72 hours following cell perturbation (Okayama *et al.,* 1995, 2003). These cytokines and chemokines undoubtedly contribute to the ongoing inflammatory response in

asthma and may be partly responsible for the allergen induced late-phase inflammatory response characteristic of allergen provocation.

Eosinophils

A very prominent cell in the inflammation of allergic asthma is the eosinophil leukocyte, which is present not only in the airway wall but in uncontrolled asthma is also found in large numbers in the sputum and bronchoalveolar lavage fluid (Kay 2005; Lemiere *et al.,* 2006). These cells are in large part initially recruited from the bone marrow as CD34 precursors, following the release of PGD2, cysteinyl leukotrienes, chemokines, and cytokines from the asthmatic airway. The developing eosinophils then pass from the circulation via the microvascular compartment into the airway wall. There has been research on the mechanisms involved in this recruitment, including the role of specific adhesion molecules and chemokines. Suffice to say that IL-3 and GM-CSF and eotaxins 1–3 are crucial to the early derivation of eosinophils from CD341 bone marrow precursor cells, with IL-5 being responsible for their maturation and recruitment into the airways (Robinson *et al.,* 1999; Sehmi *et al.,* 2003).

Eosinophils are a rich source of granule basic proteins, such as major basic protein, eosinophil cationic protein and eosinophil peroxidase, and also have the ability to generate eicosanoids such as prostacyclin (PGI2) and cysteinyl leukotrienes and release potentially tissue-damaging superoxide and a range of chemokines and cytokines (Kariyawasam and Robinson 2006). Without doubt, eosinophils play an important role in the allergic inflammatory response, but recently their primacy in the inflammatory of asthma has been challenged (Flood-Page *et al.,* 2003a). Following allergen challenge in moderately severe asthma, the administration of a humanized blocking IgG monoclonal antibody directed to IL-5 resulted in a dramatic reduction (480%) in circulating and sputum eosinophils. However, this was not accompanied by any inhibitory effect on the allergen-

induced late-phase reaction in the airways or skin or acquired airway hyper-reactivity (Leckie *et al.,* 2000; Phipps *et al.,* 2004).

Monocytes and Macrophages

Monocytes can differentiate into macrophages and dendritic cells in the presence of GMCSF (Gajewska *et al.,* 2003), the latter requiring IL-4 (Webb *et al.,* 2007). In chronic asthma both monocytes and macrophages are prominent cells in the airway mucosa and undoubtedly play an important role in disease pathogenesis. While these cells are crucial source of reactive oxygen, cysteinyl leukotrienes, and a variety of lysosomal enzymes, their precise role in mediating tissue damage and contributing to the overall airway pathology of asthma is largely unknown. In corticosteroid-refractory asthma, monocytes and macrophages are thought to play an increasingly crucial role and may well account for the ongoing chronic inflammation associated with their preferential infiltration into the airway wall in patients with longstanding corticosteroid-resistant disease (Sher *et al.,* 1994; Loke *et al.,* 2006).

Basophils

Although basophils have largely been known of as circulating IgE-triggered inflammatory cells, in certain types of immune response they do accumulate in tissues. The discovery of unique basophil-specific markers such as basogranulin (Mochizuki *et al.,* 2003; Agis *et al.,* 2006) has enabled their identification in the airways of subjects with asthma (Macfarlane *et al.,* 2000; Kepley *et al.,* 2001). However, at this time, it is not clear what their precise function is in either acute or chronic disease, although it is known that they share many of their recruitment mechanisms with eosinophils and are likely to be accompaniments of eosinophil infiltration (Gangur *et al.,* 2003)

1.1.9 Pathophysiology of Ovalbumin-Induced Asthma

Despite different pathophysiological mechanisms in allergen-induced asthma, ovalbumin (OVA) induced classical eosinophilic asthma is common. Moreover, ovalbumin in combination with endotoxin (lipopolysaccharide, LPS) at different sensitization and challenge schedules were used to induce neutrophilic and mixed-granulocytic asthma. (Simpson *et al.,* 2006; Schülke, 2019). Although ovalbumin at high doses is sufficient to induce asthma, the use of endotoxin and/or adjuvant can help exacerbate the ovalbumin - induced immune response. For this, several sensitization and challenge (acute or chronic) protocols have been developed and used. The use of the endotoxin (LPS) and adjuvant (Alum) may overcome the tolerogenic issues of ovalbumin. Moreover, the use of endotoxin in the protocols are preferred in studies focusing on acute neutrophilia. Similarly, an adjuvant such as Alum can be used to induce acute asthma models. Of note, Alum induces Th2based immune responses, which synergize and facilitate ovalbumin - induced immune response (Fig 1.2C). Nevertheless, the inclusion of an adjuvant is not necessary for some type of allergy models such as food-induced allergy models.

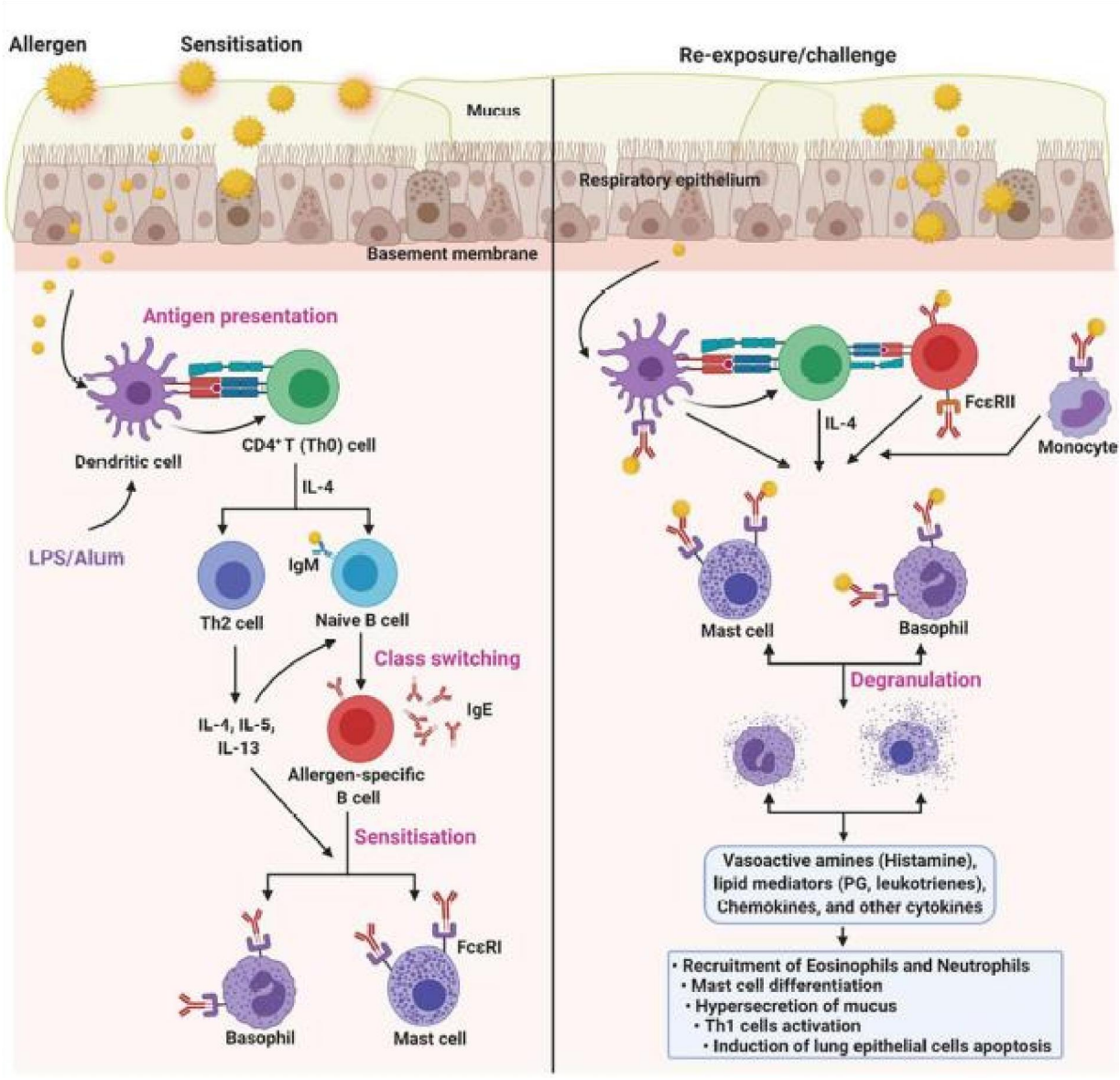


Fig 1.1.8: Pathophysiology of OVA-induced asthma. (Azman *et al.,* 2021)

Ovalbumin-induces different types of allergic asthma, including neutrophilic,eosinophilic, and mixed granulocytic asthma. Although different sensitization and challenge protocols have been used, the underlying mechanism is nearly similar. Dendritic cells (DCs) are the first innate immune cell that encounters the allergen after crossing the respiratory epithelium. DCs process and present the antigen to the CD4+ T cells, which subsequently polarized into Th2 cells via Th2 inducing factor (IL-4). Th2 cells secrete copious amounts of cytokines (IL-4, IL-5, and IL-13),

which later activate the naive B cells to antigen-specific B cells (class switching). Further, antigen-specific B cells secret IgE, which is sequentially recognized by the FcεRI on the mast cells and basophils. This process is called sensitization. Upon the re-exposure, allergen experienced leukocytes accelerate the priming process and activate basophils and mast cells. In addition, sensitized mast cells and basophils directly recognize the IgE antigen conjugates and undergo degranulation, which releases vasoactive amines (histamine), lipid mediators (leukotrienes, prostaglandin), chemokines, and other cytokines. As a total effect, the former mediators recruit the eosinophils and neutrophils, induce the mast cell differentiation, Th1 cell activation, lung epithelial cells apoptosis, hypersecretion of mucus, and others (Larché *et al.,* 2006) Of note, few protocols use LPS or Alum, which accelerate the process, to induce a specific type of allergic condition.

1.1.10 Malondialdehyde (MDA)

Malondialdehyde (MDA) is the organic compound and colorless liquid. Malondialdehyde is a highly reactive compound that occurs as the enol. It occurs naturally and is a marker for oxidative stress. Malondialdehyde results from lipid peroxidation of polyunsaturated fatty acids. It is a prominent product in thromboxane A2 synthesis wherein cyclooxygenase 1 or cycloxygenase 2 metabolizes arachidonic acid to prostaglandin E2 by platelets and a wide array of other cell types and tissues. This product is further metabolized by thromboxane synthase to thromboxane A2, 12-hydroxyheptadecatrienoic acid, and malonyldialdehyde. Alternatively, it may rearrange non-enzymatically to a mixture of 8-cis and 8-trans isomers of 12-hydroxyeicosaheptaenoic acid plus malonyldialdehyde. The degree of lipid peroxidation can be estimated by the amount of malondialdehyde in tissues. Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde. This compound is a reactive aldehyde and is one of the many reactive

electrophile species that cause toxic stress in cells and form covalent protein adducts referred to as advanced lipoxidation end-products (ALE), in analogy to advanced glycation end-products (AGE). The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism. Malondialdehyde reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts, the primary one being M1G, which is mutagenic. The guanidine group of arginine residues condense with malondialdehyde to give 2-aminopyrimidines. Human ALDH1A1 aldehyde dehydrogenase is capable of oxidizing malondialdehyde (Pryor and Stanley, 1975).

1.2 Aim of Study

Due to the mainstay of Histamine challenge epidemics and ongoing research with traditional herbal plants to better manage the disease, the aim of this study was to investigate the effect of ethanol extract of *Solanum nigrum* on the concentration of malondialdehyde (MDA) after histamine challenge in ovalbumin sensitized guinea pigs

1.3 Objective of Study

The objective of this research is to consider the histopathological part of this research, thus ascertaining possible activities of Solanum *nigrum* against inflammation in the lungs of guinea pigs induced with ovalbumin as a model for allergy such as asthma.

CHAPTER TWO

2.0 METHODOLOGY

2.1 Collection of Plant Material

The plant materials were obtained from around the environment of the Department of Biochemistry, University of Benin. Identification and botanical authentication of the plant was done by Dr. Akinibosun of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. A specimen was deposited in the same Department's herbarium with a voucher number UBHs320.

2.1.1 Animals

Three weeks old, male guinea pigs, weighing (375-380) g, were obtained from the Laboratory of Animal Centre, Edo State University, Ekpoma, Nigeria. The animals were maintained under standard environmental conditions (12-hour light and dark cycle) with free access to standard animal chow and water *ad libitum* for 2 weeks, prior to the commencement of the study. Approval for the use of animals for the study was obtained from the Ethical Committee on the Use of Laboratory Animals, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Animals were handled according to the protocol outlined in "Principles of Laboratory Animal Care" (National Institute of Health Guide for Care and Use of Laboratory Animals, Pub No. 85 – 23, revised 1985).

2.2 Preparation of ethanol extract of *S. nigrum*

Leaves from the young shoot were collected from the quadrangle in the Department of Biochemistry, University of Benin, Benin City while two thousand, four hundred grams (2400g) of the wet leaves was weight out for air drying. The leaves were air dried at 31^{o} C for four weeks, after which it was pulverized using a versatile laboratory blender 120v, 50/60Hz (waring product). The powdered leaves (500 g) were extracted using two (02) Winchester bottles of two and a half Litres (2.5 L) each, of a 95% ethanol as solvent in Soxhlet extractor. The ethanol extract was concentrated using a rotary evaporator (Büchi, Rotavapor R-200), the resulting concentrate was lyophilized at -70^{o}C using SCIENTZ-50F vacuum big LCD display heating function freeze dryer. The resultant solid extract was stored in a refrigerator at 4^{o}C for forty-eight hours before used.

2.3.1 Sensitization

To evaluate anti-asthmatic activities, all sensitization were by the ovalbumin (ova) sensitized Guinea pig model of asthma. Briefly the method of Bramley *et al* (1995), was used, with minor modification as follows; the animals were sensitized with ovalbumin in 0.9% saline solution by injecting of 100 mg/ kgbw ovalbumin (ova) on day one intraperitoneally, followed by 100 mg/kgbw booster doses, 24 and 48 hours later

2.3.2 Induction of bronchospasm in conscious guinea pig (characteristics properties of Asthma)

This study was carried out according to the method previously described by Armitage *et al*., (1961), modified by Kumar, (2011).

Principle

Histamine induced broncho-constriction is the traditional immunological model of antigen induced airway obstruction. Histamine aerosol when inhaled causes hypoxia and leads to convulsion in guinea pigs and causes very strong smooth muscle contraction, profound hypotension, and capillary dilation in cardiovascular system. A prominent effect caused by histamine leads to severe broncho-constriction in the guinea pigs that causes asphyxia and convulsions resembling bronchial asthma. Pre-convulsive time (PCT), is a measure of the time between the exposure of animals to histamine aerosol to the onset of dyspnoea leading to the appearance of convulsion. The occurrence of these symptoms can be delayed by bronchodilator drugs.

Procedure

Twenty-five (25) ovalbumin sensitized guinea pigs fasted overnight were used with five (05) animals per group. Bronchospasm was induced in guinea pigs by exposing them to histamine aerosol (2%) produced by an ultra-sound nebulizer at a pressure of 300 mm Hg in an aerosol chamber (24 x 14 x 24 cm, made of perplex glass). The time required for appearance of pre-convulsive dyspnoea caused by the histamine was recorded for each animal. Prior to drug treatment, each animal was placed in the histamine chamber and exposed to 2 % histamine

aerosol. The pre-convulsion time (PCT), i.e. the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion, was noted. As soon as the pre-convulsion dyspnoea (PCD) was noted, the animals were removed from the chamber and placed in fresh air to recover. This time for pre-convulsive dyspnoea was recorded as basal value. Guinea pigs were then allowed to recover from dyspnoea for 24 hrs. After 24 hrs, the animals of groups 2, 3, 4 received 50mg/kgbw, 100mg/kgbw and 200mg/kgbw ethanol extract of *S. nigrum*, p.o and group 5 received the reference drug aminophylline (25mg/ kgbw, i.p). These animals were again subjected to histamine aerosol later after 1hr of administering drugs, to determine pre-convulsion time (PCT). The protection offered by the treatment was calculated by using the following formula

Calculation:

Percentage protection = (1-T1/T2) X 100 (Singh, 1990)

Where T1 = mean of PCT before administration of test drugs, and T2 = mean of PCT after administration of drugs

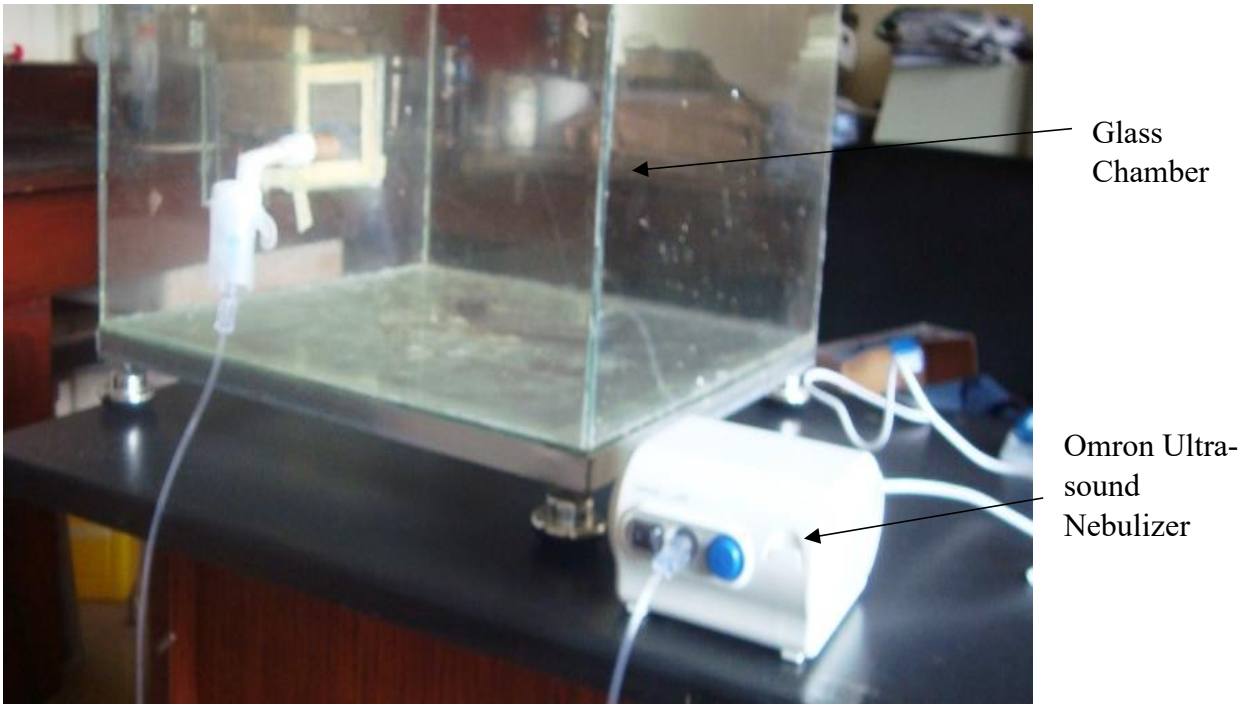


Fig. 2.3.2. Aerosol chamber for inducing allergic asthma

2.4 Early and Late Airways Response

Principle

Early airways response (EAR) reaches a peak at about 5-30 min after challenge and lasts for 2 hours, and in more than 50% of atopic asthmatic patients, the EAR is followed by the Late airways response (LAR), which occurs between 1-12 h after challenge and lasts for several hours (Hargreave, 1989).

Procedure

Forty (40) guinea pigs were sensitized in this experiment, though forty-five guinea pigs were used. The following treatment schedule was adopted:

The first group of 5 animals received only food and water for 21 days, while second group was sensitized in addition to food and water *ad libidum*. The third group in was had free access to food and water, sensitized and expose to histamine aerosol on 21 day. The fourth group in addition to food and water also received ethanol extract of *S. nigrum* leaves (100 mg/kg) from day 1 of sensitization till day 21, when they were exposed to histamine aerosol. The fifth group received the extract (200 mg/kg) two days after last sensitization till day 21 and the exposure to histamine aerosol. The sixth group was sensitized (20 animals in all) and left untreated with ethanol extract but given food and water *ad libidum.*

After 21 days, the extract- treated guinea pigs were placed in a glass chamber and exposed to histamine (2%) aerosol produced by an ultra-sound nebulizer (Omron) under a mean pressure of 280 ± 5mmHg till the first signs of pre-convulsive breathing. Five hours later, the animals were sacrificed, blood, and bronchi alveolar lavage (BAL) fluid, were collected for biochemical and hematological analysis. Sections of the tracheal and the lungs excised for biochemical and histopathological analysis.

The sensitized animals in the untreated group were randomly placed into 4 different groups of 5 animals each as follows:

Group 1 (control): Distilled water + histamine aerosol (2%)

Groups 2 –3: 100 and 200 mg/kg of the ethanol extract, respectively + histamine aerosol (2%)

Group 4: aminophylline (25mg/kg, ip) + histamine aerosol (2%)

Aminophylline and ethanol extract were administered i.p and p.o one hour respectively prior to aerosolization. Once extracted from the respiratory chamber, each group members were housed

for a further 2 hours and eventually sacrificed. The sensitized guinea pigs of group 2, which were excluded from aerosol administration, underwent the same fate with blood collected and tracheal flush with normal saline to obtain the bronchi alveolar lavage (BAL) fluid for biochemical and hematological analysis.

2.5 Sample Collection

Lung and tracheae were dissected out and washed immediately with ice cold normal saline to remove as much blood as possible. Lung and tracheae homogenates (5% w/v) were prepared in cold 50 mM Tris buffer (pH 7.4) using mortal pestle with acid wash sand to act as abrasive. The homogenate obtained was centrifuged at 3000 rpm for 10 min and the resultant supernatant used for the biochemical assays. Blood samples were collected in 3mL tubes that contained heparin as anticoagulant. The heparin tubes were centrifuged at 3500 rpm for 15 min to obtain plasma also used for biochemical analysis.

2.5.1 Bronchoalveolar lavage fluid (BALF):

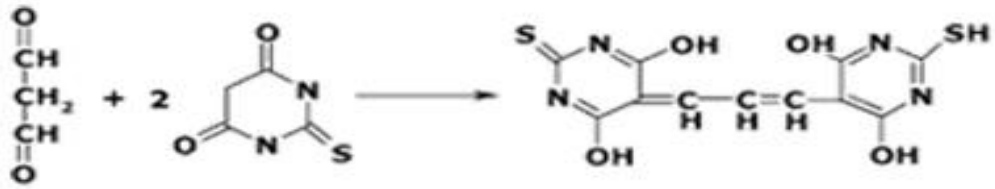
Lung lavage was performed gently using 3 mL of PBS (phosphate-buffered saline) at 37°C. The recovered lavage samples were cooled on ice and centrifuged at 150 × g for 10 min, the resultant supernatant was used for biochemical analysis.

2.6 Estimation of malondialdehyde (MDA) concentration

The assay method of Buege and Aust (1978), was adopted.

Principle

Malondialadehyde is formed from the breakdown of polyunsaturated fatty acids and it serves as a convenient index for the determination of the extent of peroxidation reaction. Malondialdehyde (MDA), a product of lipid peroxidation, when heated with the chromogenic reagent, 2-thiobarbituric acid (TBA) under acid conditions forms a pink coloured product as shown in the equation below with a maximum absorbance at 532 nm.



MDA TBA pink colored product

Procedure

Three milliliters each of (1:1:1v/v/v) TCA-TBA-HCl reagent were added to test tubes appropriately labelled blank and tests. Distilled water (0.5 ml) was added to the blank, while 0.6 mL of the appropriate tissue extract was added to each of the sample tubes. These were thoroughly mixed, incubated in a boiling water bath for 15 minutes then allowed to cool, after which they were centrifuged and their supernatants collected. The supernatant from the blank was used to zero the spectrophotometer (preset at 532 nm) before reading the absorbance of the supernatants from the test solutions.

Calculation

Concentration of MDA in the tissue (nmoles MDA/mg protein) =

$$\frac{\text{Absorbance of sample}}{A \times l \times p}$$

Where:

A = Molar extinction coefficient of the product and according to Buege and Aust (1978), is equal to $1.56 \times 10^5\ M^{-1}\ cm^{-1}$

p = protein concentration of sample used

2.6.1 Total Protein

The total protein was determined using randox total protien kit (Biuret method based on George and o' Neil, 2001).

Principle

The peptide bonds of protein react with biuret in an alkaline medium to give a purple colour. The intensity of the colour which is measured at 540-570 nm is proportional to the protein concentration.

Procedure

Three text tubes namely; reagent blank, standard and sample contain 0.02 mL each of sample solution respectively. (Biuret reagent) was added to all the test tubes, vortexes then allowed to

stand at 31°C for 30 min and the absorbance of sample tube and the standard tube were read at 546 nm against the reagent blank.

Calculation:

$$\text{Concentration of protein} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{standard concentration (5.78 g/dl)}$$

CHAPTER THREE

3.0 RESULTS

Data obtained from this study was analysed using the Graph-Pad prism statistical software (Version 9.11). One-way analysis of variance (ANOVA) was used to compare means, followed by the Tukey's test correction and values were considered significant at $P< 0.05$. All the data are expressed as mean ± Standard error of the mean (SEM).

In the early stage of asthma, release of inflammatory mediators like histamine, acetylcholine, leukotrienes, and prostaglandins are triggered by exposure to allergen. This mediator induces broncho-constriction. Inhalation of histamine is a classical model of inducing broncho-constriction, in the guinea pig airways; exposure to histamine aerosols results in intense smooth muscle contractions, hypoxia leading to convulsion, asphyxia and death. Bronchodilators can delay the occurrence of these symptoms (Nayampalli *et al.,* 1986). The end points pre-convulsion dyspnea (PCD) was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsion.

Table below shows the effect of pre-treatment of ova-sensitized guinea pigs with normal saline (control), 50, 100, 200mg/kg bw *S. nigrum* ethanol leaf extracts and a standard drug (25mg/kgbw aminophylline) on the time taken for histamine-induced pre-convulsive dyspneoa to occur. There was a significance time difference between the animals administered normal saline and 50 mg/kgbw *S. nigrum* extract ($P < 0.05$). This time difference increase as the concentration of the extract increase from 50mg/kgbw to 200mg/kg bw progressively, though there was no significant difference in time between the animals administered 100mg/kgbw and those administered 200mg/kgbw. The percentage increase in time was highest with the standard drug 25mg/kgbw

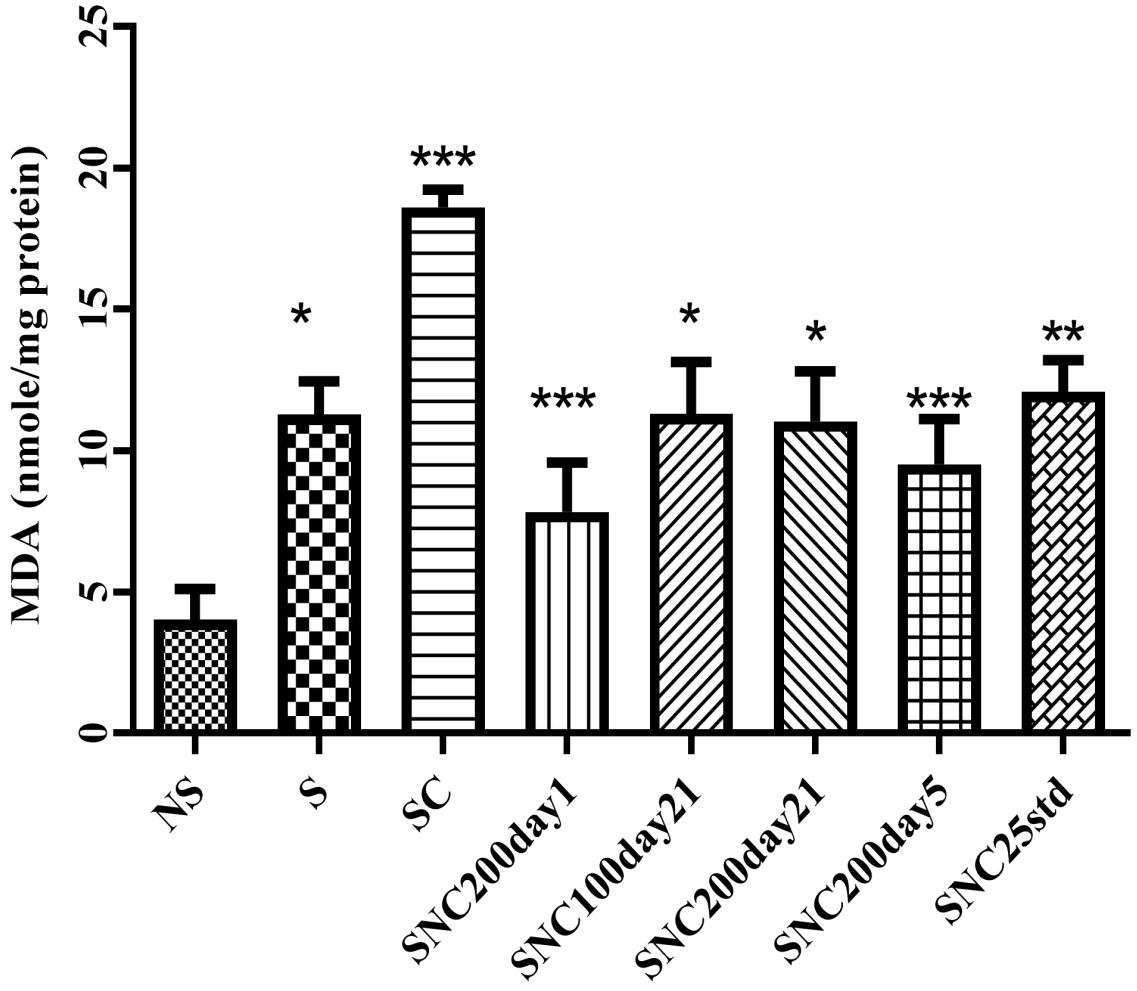
aminophylline, 61.71%. *Solanum nigrum* showed a dose dependent protection against histamine-induction bronco-constriction by of convulsion at all doses administered when compare to control (normal saline) significantly prolonging the latent period of collection at all doses administered when compared to control (normal saline) following exposure to 2% histamine aerosol.

Effect of ethanol extract of *S. nigrum* leaf on histamine induced broncho-constriction in guinea pigs

Groups	Pre-convulsion Dyspnea Time (Sec.)		
	Before treatment (Control)	After treatment	% Increase in the time of PCD
Normal saline	182.80 ±5.51	181.60 ±6.04	0.00
50mg/kgbw extract	154.60 ±9.13	275.00 ±17.31*	43.78
100mg/kgwb extract	131.00 ±2.40	286.40 ±10.85**	54.26
200mg/kgbw extract	209.60 ±10.8	501.60 ±33.05***	58.21
25mg/kgbw std drug (Aminophylline)	194.20 ±4.8	507.20 ±24.89***	61.71

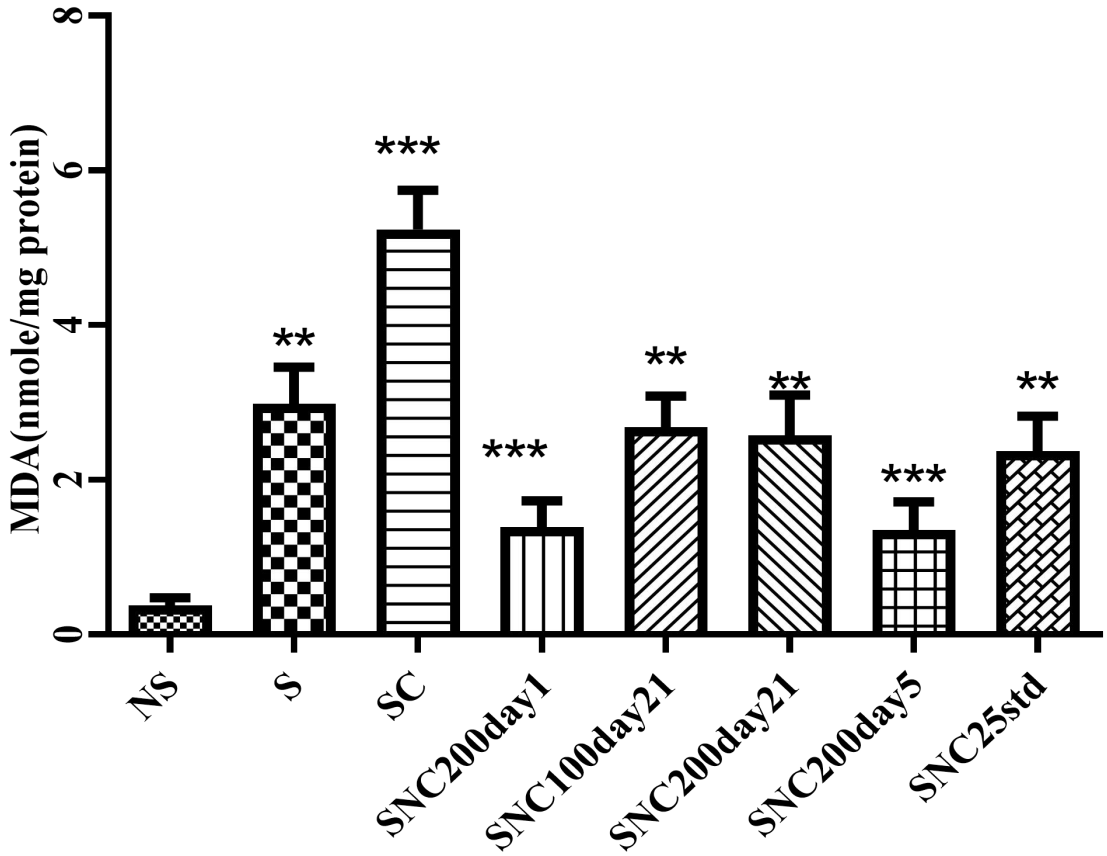
Each value was expressed as mean ± S.E M, where n = 5 at *P< 0.05, ** P < 0.01, *** P< 0.001. The effects of *S. nigrum* ethanol extract on lipid peroxidation resulting from asthmatic attack, as represented in this study by the sensitization and challenge of guinea pig is shown in figures below lipid peroxidation in bronchial alveolar tissue, lung and plasma cells respectively. The

results showed that animals in SC group had a high level of lipid peroxidation represented by malondialdehyde concentration, when compared with control, the non- sensitized group (NS) $P< 0.001$. Treatment with the extracts at various concentration and duration period there was a drastic drop in the concentration of malondialdehyde, indicating a reduction in lipid peroxidation resulting from asthmatic attacks. The groups in SNC200day1 and SNC200day5 were comparable with SNC25std with no significant value between them. But when these groups are compared with SC group where malondialdehyde is highest, there is a significant reduction due to treatment $P < 0.001$. Meanwhile there was no significant different between these groups and NS group (control), ie the extract when giving at 200gm/kgbw early enough will prevent the lipid peroxidation of individual under asthmatic attack. But if given at the onset of the attack the chances of prevention is slim, because on day 21 treatment there was minimal effect as shown by a decrease in the $P < 0.05$ for SCN200day21 and SNC100day21 values when compared with SC group.



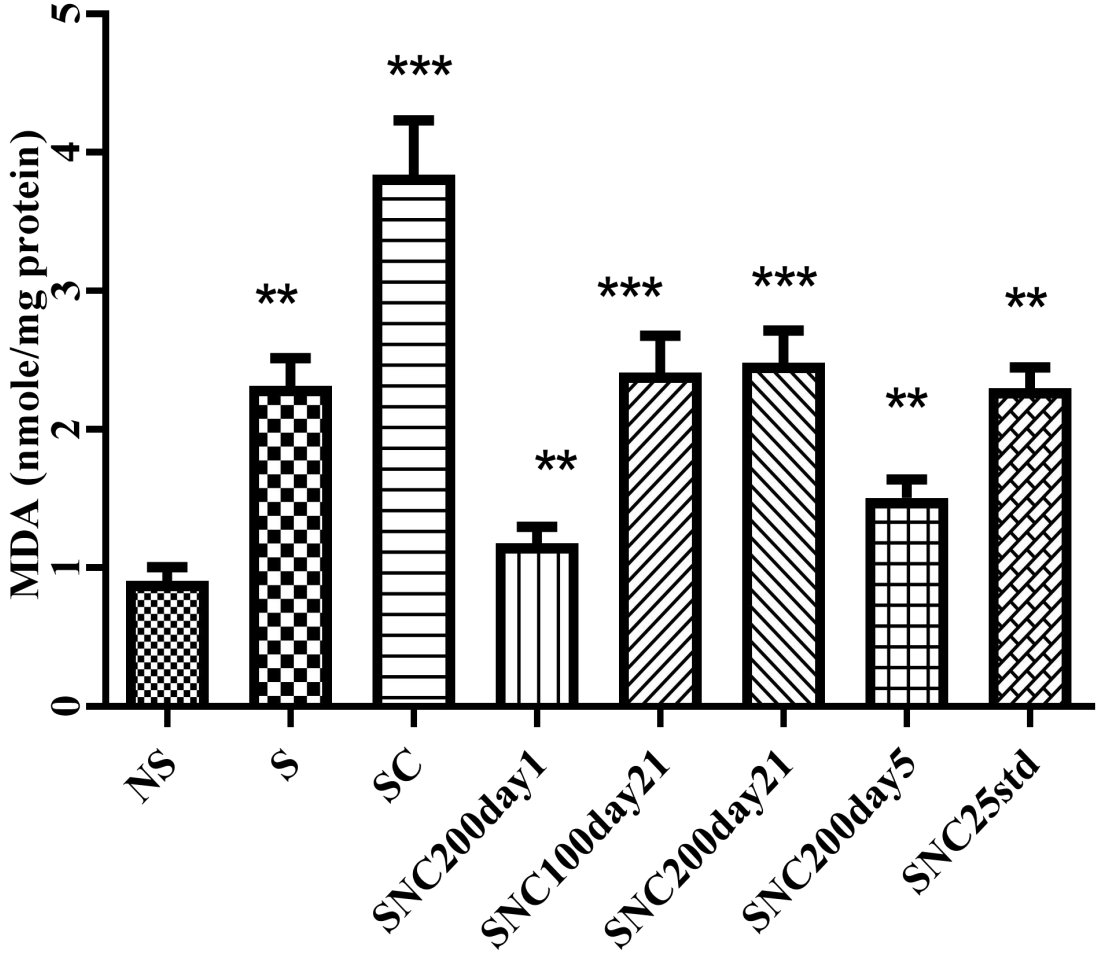
The effect of ethanol extract of *S. nigrum* on the concentration of MDA in the bronchial alveolar lavage fluid after histamine challenge in ovalbumin-sensitized guinea pigs BALF

Each column represents the mean ± SEM of, concentration of MDA (for each group n = 5), Statistical differences between NS and S *P< 0.05., NS and SC ***P< 0.001., SC and treated groups.* P< 0.05)., **P< 0.01.,***P< 0,001



The effect of ethanol extract of *S. nigrum* on level of Malondialdehyde in the lungs after histamine challenge in ovalbumin-sensitized guinea pigs

Each column represents the mean ± SEM of, concentration of MDA (for each group n = 5),. Statistical differences between NS and S *P< 0.05., NS and SC ***P< 0.001., SC and treated groups.* P< 0.05)., **P< 0.01.,***P< 0,001



The effect of ethanol extract of *S. nigrum* on MDA in the plasma after histamine challenge in ovalbumin-sensitized guinea pigs

Each column represents the mean ± SEM of, concentration of MDA (for each group n = 5), Statistical differences between NS and S *P< 0.05., NS and SC ***P< 0.001., SC and treated groups.* P< 0.05)., **P< 0.01.,***P< 0,001

CHAPTER FOUR

DISCUSSION AND CONCLUSION

DISCUSSION

Ethanol extract of *Solanum nigrum L* can be used to treat asthma and it observed to show a protective effect against asthma. Asthma which is a disease causes the release of inflammatory mediators like histamine, acetylcholine leukotrienes and prostaglandins which are triggered by exposure to allergen in the early stage. These mediators cause a lot of damages to the body cells, such as the release of reactive oxygen species (ROS), lipid peroxidation which are toxic to the cell. The guinea pig tracheal chain preparation is widely accepted in in vitro model employed to screen the potential efficacy of compounds like antihistamines, leukotriene receptor antagonists, cholinergic antagonists and 2- adrenergic receptor agonists (Muccitelli et al., 1987). It has been observed in the present study that the ethanol extract of *S. nigrum* employed at various doses did produce protected effect prior to the exposure to histamine. It shows the effect of pre-treatment of ova-sensitized guinea pigs with normal saline (control), 50, 100, 200mg/kgbw *S. nigrum* ethanol leaf extracts and a standard drug (25mg/kgbw aminophylline) on the time taken for histamine-induced pre-convulsive dyspneoa to occur, it was observed that there was delayed response. There was a significance time difference between the animals administered normal saline and 50 mg/kgbw *S. nigrum* extract ($P < 0.05$). This time difference increases as the concentration of the extract increases from 50mg/kgbw to 200mg/kgbw progressively, though the difference in time between the animals administered 100mg/kgbw and those administered 200mg/kgbw was not significant. Although the percentage increase in time was found to be highest with the standard drug 25mg/kgbw aminophylline, 61.71%. It can then be concluded that *Solanum nigrum* showed a dose dependent protection against histamine-induced bronco-constriction by delaying the time taken for the development of pre-convulsion dyspnea at all doses administered when compared to control (normal saline).

The increase in the concentration of malondialdehyde is one of the most frequently used indicators of lipid peroxidation of the membranes which is as a result of oxidative damage i.e the breakdown of poly unsaturated fatty acids. From the result gotten the effect of ethanol extract of *S. nigrum* on the concentration of MDA in the bronchial alveolar lavage fluid, lungs and plasma

after histamine challenge in ovalbumin-sensitized guinea pigs BALF, it was observed that animals that are sensitized by ovalbumin and then exposed to histamine aerosol showed a high level of lipid peroxidation representing high concentration of MDA in the bronchial alveolar lavage fluid, lungs and plasma when compared the control (NS). With the treatment with various concentration of ethanol extract of *S. nigrum*, there was a decrease in the concentration of MDA indicating a reduction in lipid peroxidation that resulted from asthmatic attack. The MDA concentration of groups in SNC200day1 and SNC200day5 were comparable with SNC25std with no significant difference between them. But comparing these groups with SC group where malondialdehyde is highest, there is a significant reduction due to treatment $P < 0.001$. Meanwhile there was no significant difference between these groups and NS group (control), i.e the extract when giving at 200gm/kgbw early enough will prevent the lipid peroxidation of the animals under asthmatic attack. But if treatment was not given at the early attack the chances of prevention is slim, because on day 21 treatment there was minimal effect as shown by a decrease in the $P < 0.05$ for SCN200day21 and SNC100day21 values when compared with SC group. This is in agreement with Inas R. *et al*., who reported that the serum concentration of MDA was highly significantly increased in the studied patients compared to controls ($P < 0.001$), and in severe asthma compared to the studied patients with moderate and mild asthma ($P < 0.001$).

This decrease in the concentration of malondialdehyde (MDA) is an indication of reduction in the peroxidation of tissues.

CONCLUSION

Asthma can be induced in guinea pigs and these animals are very sensitive to it. The asthma can then be treated with *Solanum nigrum Loca* which has preventive and protective effects against asthma.

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