OPEN ACCESS
Scholars International Journal of Traditional and Complementary Medicine
Abbreviated Key Title: Sch Int J Tradit Complement Med
ISSN 2616-8634 (Print) |ISSN 2617-3891 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Review Article

Therapeutic Potential of *Azadirachta indica* (Neem)-A Comprehensive Review

Hafiza Anam Asghar[1], Syed Qamar Abbas[1], Muhammad Kamran Arshad[1*], Asra Jabin[1], Bakhtawar Usman[1], Mueez Aslam[1], Azeem Asghar[1]

[1]National Institute of Food Science & Technology, Faculty of Food, Nutrition and Home Sciences, University of Agriculture Faisalabad, Pakistan.

DOI: 10.36348/sijtcm.2022.v05i03.001 | **Received:** 28.01.2022 | **Accepted:** 03.03.2022 | **Published:** 08.03.2022

***Corresponding author:** Muhammad Kamran Arshad
National Institute of Food Science & Technology, Faculty of Food, Nutrition and Home Sciences, University of Agriculture Faisalabad, Pakistan

Abstract

Background: Plant based traditional health care is one of the ancient remedies used to prevent and treat many health-related disorders. Due to increasing cost of medicine in the modern era, people are now moving towards the utilization of ancient ethno medicinal plants-based remedies to prevent and treat diseases as well as to maintain their health. *Azadirachta indica*, commonly known as neem has been used since ancient times as ethno medicinal plant due to its pharmacological and therapeutic potential. Different parts of this plant including leaves, fruit, bark, seeds and flowers can be used to prevent many diseases. Many structurally complex and biologically active components can be extracted and utilized from different parts of neem. ***Purpose:*** Purpose of this review is to highlight the importance of different parts of neem as they contain various biologically active components that are beneficial in prevention and treatment of various health related disorders. ***Method:*** Science direct, Google scholar, Web of Science and PubMed were accessed to review the therapeutic potential of neem in different diseases. ***Results:*** Neem has been demonstrated to exhibit anti-cancer, immunostimulant, orodental, skin protection, neuropharmacological, ulcer treating, anti-pyretic, anti-inflammatory, anti-malarial, anti-bacterial, anti-fungal, anti-viral, anti-parasitic, anti-hyperglycemic, antioxidant, anti-hyperlipidemic, hepatoprotective, renal protection and hematological parameters maintaince properties. ***Conclusion:*** There is no evidence of adverse effects of neem in literature. Only the people who are allergic to it can have side effects otherwise it is stomach friendly in all conditions due to which it can be used for treatment of various health related disorders.

Keywords: Plant based medicine, *Azadirachta indica*, neem, therapeutic potential.

INTRODUCTION

Plant based health care treatment against health-related human disorders is as old as human civilization. Worldwide, traditional health care systems mainly include plants as major source of medicines. Now days, plant-based medicines are also being used in developed as well as developing countries. It is estimated that on Earth higher plant species are almost 250,000 but biological activities are observed only in 6% plants and phytochemically characterized plants are 15%. Exponentially, demand of natural products as well as synthetic drugs is increasing globally (Villasen and Lamadrid, 2006).

Azadirachta indica (Neem) has been widely used in modern medicine, homeopathy, unani and ayurveda. Various parts of neem contain more than 140 biologically active components that have complex structure and diverse chemical nature. Every part of neem tree including bark, roots, fruits, seeds, flowers and leaves have been traditionally used for prevention and treatment of various health disorders such as dental diseases, skin diseases, fever, infections and inflammation. Medicinal properties of neem have been mainly referred to the leaves of neem. Neem leaves as whole and active components from its leaves have been widely used for its multiple health effects including anticarcinogenic, antimutagenic, antioxidant, antiviral and antibacterial, antifungal, antimalarial, antiulcer, antihyperglycemic, anti-inflammatory and immunomodulatory (Subapriya and Nagini, 2005). Therefore, ethno medicinal plants should be included in the daily diet to prevent lifestyle related disorders and decreasing the expenditure on modern medicine.

Plant Based Traditional Health Care

Traditional Chinese medicine, traditional Indian medicine and Ayurveda are the current and

ancient traditions used in medicine. With the passage of time, public interest is increasing in complementary and alternative medicine due to increased rate of emerging diseases, resistance to microbes, increased cost of new and emerging drugs, lack of treatment against chronic diseases and increased side effects of allopathic medicines (Humber, 2002).

Alternative therapeutic approaches have been used by 2/3rd of United States population by 2010. As traditional medicines and dietary supplements, more than 1500 herbs of natural origin are being used as part of indigenous drug therapy. Due to this, pharmaceutical companies used these drugs as part of natural therapy. Trade of raw materials, botanical drug products and medicinal plants has increased from 5-15% (WHO, 2000).

Traditional Chinese medicine and ayurveda have been used from long time for prevention and treatment of diseases and improving the quality of life. An essential requirement for effective and safe use of therapeutic agents is consistency in biological activity and composition of botanical drugs. Efficacy and safety of botanical medicines is critically determined by quality of botanical drugs. Rare formulations, drugs, extracted raw materials and botanical preparations (Cardellina, 2002).

Literature showed that for diabetic patients, more than 400 various plant extracts and plants have been utilized that show significant hypoglycemic activity. Medicinal plants that have hypoglycemic activity were classified into three basic categories: Firstly, plants from which active ingredient having hypoglycemic activity was extracted and evaluated. Secondly, plants having hypoglycemic activity but active ingredient responsible for glucose lowering action were unknown and not characterized. Thirdly, plants having hypoglycemic activity, but scientific evidence are not present (Bailey and Day, 1989).

In the history, some ayurvedic products are used in the prevention and treatment of some common diseases such as childhood diarrhea, nutritional deficiencies, lactation difficulties, abdominal complications, postpartum problems such as uterine complications and pain, edema during pregnancy and anemia (Patwardhan *et al*., 2005).

In addition to existing anti-diabetic treatments, some traditional anti-diabetic plants are now being considered important for production of new pharmaceuticals by using traditional anti-diabetic plants as an important source of new and emerging oral hypoglycemic agents. Plant based molecules are considered as treatment for diabetes because they promote the action of insulin or increase the secretion or endogenous biosynthesis of insulin Personal experience of population and cumulative knowledge showed that consumption of plants or plant constituents over many years as a part of diet may cause detrimental effects on health but people are using natural treatments due to their effectiveness. Due to these reasons, traditional anti-diabetic plants should be consumed with care (Bailey and Day, 1989). As the use of plant based therapy is an ancient civilization, it must be done with proper care because many plants or parts of plants may contain some harmful components that may be detrimental to health. So, further research must be conducted on medicinal plants for the purpose of isolating and utilizing their bioactive components for human health purposes.

Azadirachta indica (Neem): An overview

Medicinal plant is defined as a plant having pharmacological action to treat any disorder in comparison to other plants that are mostly used as food. Neem has been used for more than 2000 years as medicinal plant. Due to high biological activities it is used as versatile medicinal plants. Various neem parts have structurally complex and diverse components (Subapriya and Nagini, 2005).

Neem is considered a cynosure of modern medicine because it is used abundantly in homeopathic, unani and ayurvedic medicine. For treating various human disorders, almost all parts of neem tree are used for medicinal purposes as household remedies including barks, roots, seeds, flowers and leaves. Neem leaves are available throughout the year and active ingredients can be easily extracted due to which they are mainly used in medicinal preparations (Puri, 1999).

Azadiracta indica is mainly used in complementary and alternative medicinal system, a type of conventional medicine including unani medicine, ayurveda and homeopathy. According to unani system, it is effectively used to strengthen gums and teeth, enrich blood and boost the liver. It is famous for its multiple health benefits due to its use as anti-malarial, anti-fungal, anti-microbial and anti-parasitic in various species of animals. Progression of various chronic disorders including cancer, diabetes and cardiovascular diseases can be decreased by consumption of herbs and foods rich in polyphenols and flavonoids (Babu *et al*., 2013). Different parts of neem including leaves, seeds, flowers and fruit should be used extensively in medicinal preparations due to broad spectrum of pharmacological activities mainly attributed to various biologically active components present in different parts of neem.

THERAPUTIC POTENTIAL OF NEEM

Anti-Cancer Effect

Neem is versatile medicinal plant that provides multiple health benefits from ancient times. Cancer is abnormal and uncontrolled growth of cells that will disturb the normal functions of body. It is generally considered an untreated disease but literature shows that

cancer can be cured by using neem leaves extract. A study was done in which mouth and stomach cancer was induced in rats by using MNNG. After administration of neem leaves extract mitotic activity of tumor cells was inhibited (Arivazhagan *et al*., 1999).

In blood, liver and target organs of host 7,12-dimethylbenzanthracene (*DMBA*)-*induced* hamster buccal pouch (*HBP*) *carcinogenesis was suppressed due to modulation of carcinogen metabolizing enzyme and cellular redox status by using alcoholic and aqueous neem leaves extract (*Subapriya *et al.,* 2004).

A research was done on murine models related to carcinogenesis. Aqueous neem leaves extract administration decrease tumor incidence and tumor burden in DMBA induced skin pappilomas and benzopyrene induced stomach tumors. Results of this study showed that chemoprotective activity of neem leaves extract is due to carcinogen detoxification mainly associated with activities of phase-II enzymes. Anti-cancerous effect is also due to increased antioxidant status in liver after intake of neem leaves extract (*Dasgupta et al., 2004).*

Detoxification and antioxidant enzymes were enhanced in rats having hepatocellular carcinoma by intake of 5% neem leaves extract. Administration of neem leaf extract cause significant reduction in B16 melanoma cells. Neem leaf extract is chemo protective because it inhibits the synthesis of essential metabolites and prostaglandins that are responsible for tumor production (*Baral and Chattopadhyay, 2004)*. Azadirone 1 is an active liminoid present in neem leaves which shows cytotoxic activity against prostate, melanoma and breast cancer (*Nanduri et al., 2003).*

Azadirachtin A and nimboline is effective against human glioblastoma cells as cytotoxic agents (Akudugu *et al*., 2001). Flavonoids such as kaemferol and quercitin are present in neem leaves extract that inhibit carcinogenesis at initiation and promotion phase due to free radical scavenging property (Rice-Evans *et al*., 1996).

One of the important anticancer bioflavonoids in neem leaves is quercitin that inhibit the growth of tumor cells. Therapeutic efficacy of chemotherapeutic drugs and radiations can be enhanced by using quercitin. P-450 monooxygenase system is inhibited by quercitin because this system is mainly involved in chemical carcinogenesis activation. Quercitin is also responsible for down regulation of p-21 ras oncogene and mutant p-53 gene. Quercitin is responsible for inhibition of human breast cancer cells by promoting expression of type II estrogen receptor (ER II) (Scambia *et al*., 1993). Quercitin possess wide range of biological activities due to which it is used as a strong anti-cancer agent because it has strong tyrosine kinase activity, inhibition of protein kinase C, interaction with ER II binding sites and cell cycle regulation (Lamson and Brignall, 2000).

Survival of rats was improved due to inhibition of murine B-16 melanoma after administration of neem leaves glycoprotein (NLGP). Neem leaves glycoprotein's (NLGP) are responsible for anti-tumor activity due to down regulation of suppressive cellular functions and increased activity of $CD8^+$ T cells activity. After administration of NLGP, tumor micro-environment (TME) was shifted from type 2 inflammatory cytokines such as interlukin-6 (IL-6), tumor growth factor beta (TGFβ) and interlukin-10 (IL-10) to type 1 microenvironment which was dominant in other inflammatory cytokines containing interlukin-2 (IL-2) and IFNγ. Results of study showed that NLGP inhibit the growth of melanomas by normalizing tumor microenvironment as compared sutent and cispalin to enhance the activity of T cells (Barik *et al*., 2013).

A study was performed on cell lines of breast cancer by using ethanolic extract of neem leaves that inhibit signaling pathway of insulin like growth factor (IGF) and induce apoptosis in breast cancer cells which prevent spreading of these cancerous cells (Perumal *et al*., 2012). Results of another study showed that nimbolide present in neem leaves induce apoptosis in breast cancer cell lines of humans (Elumalai *et al*., 2012).

Autophagy and p53-independent apoptosis was induced in cancerous cells after administration of liminoids from neem oil (Srivastava *et al*., 2012). Growth of cancerous cells of pancreas was inhibited after intake of neem leaves extract due to its effect on rel protein regulated cell death (Veeraraghavan *et al*., 2011). Growth of tumor was also inhibited in cancer of prostate after administration of *Azadirachta indica* (Mahapatra *et al*., 2011).

Cancer was induced in mammary glands of rats by using DMBA and effect of methanolic fraction (MF) and ethyl acetate fraction (EAF) of neem leaves was checked against mammary carcinogenesis. Incidence of tumor was effectively inhibited after administration of 10 mg/kg body weight of methanolic as well as ethyl acetate fraction of neem leaves. Chemo preventive effect of neem leaves is due to apoptosis induction, inhibiting cell proliferation, oxidative DNA damage inhibition, enhancing level of anti-oxidants, modulation of protein and lipid oxidation, enzymes for metabolizing xenobiotic and modulation of receptor status and hormones. Results of study showed that EAF was rich in phytochemicals as compared to MF (Vinothini *et al.,* 2009).

Natural killer cells (NK) including $CD56^+$ $CD3^-$ were activated after administration of neem leaf preparation that enhanced the cytotoxic ability of these NK cells against tumor cells. Administration of neem

leaf preparation also enhanced the release of interleukin-12 (IL-12) from macrophages in healthy individuals as well as in patients having neck and head squamous cells carcinoma. Neutralization of IL-12 was observed that resulted in up regulation of cytotoxic activity of natural killer cells due to decreased activity of peripheral blood mononuclear cells after neem leaves administration. Up regulated expression of CD40 in $CD14^+$ monocytes as well as up regulation of CD40L in $CD56^+$ lymphocytes was also observed after administration of neem leaf preparation. Neem leaf preparation administration basically resulted in neutralization of CD40 and CD40L that causes down regulation of IL-12 release and cytotoxicity of NK cells. This mechanism is due to activation of p38MAPK pathway but not by ERK ½ signaling pathway. It was concluded from the results of this study that neem leaf preparation affects NK cellular cytotoxicity due to CD40-CD40L dependent endogenous production of IL-12 that control tumor cell cytotoxicity (Bose and Baral, 2007).

Azadirachta indica has been used since ancient times for cancer prevention and treatment. A study was conducted on potential of neem leaves extract anti-inflammatory properties linked to nuclear factor kappa B (NF-kappaB) signaling pathway links to apoptosis, inflammation and cancer. Methanolic neem leaf extract was used to treat cultured human leukemia cells with or without stimulation of tumor necrosis factor alpha (TNF-α). NF-kappaB was inhibited due to stimulation of TNF-α by using methanolic extract of neem leaves which proved that neem leaf extract has potential for apoptotic cell death mechanism and pro-inflammatory signaling pathway (Schumacher *et al*., 2011).

Neem is effective in cancer prevention and treatment because of some biologically active components that are responsible for stimulation and inhibition of several pathways involved in cancer progression. Neem is responsible for inhibiting the mitotic activity in many tumor cells, modulation of carcinogen metabolizing enzyme, improving antioxidant status of the body, *inhibiting the synthesis of essential metabolites and prostaglandins that are responsible for tumor production, inhibition of* P-450 monooxygenase system, down regulation of p-21 ras oncogene and mutant p-53 gene, normalizing the tumor microenvironment due to presence of neem leaves glyco proteins. Neem extracts are also responsible for inducing apoptosis in tumor cells resulting in the death of these cells and preventing the cancer progression, modulating the activity of natural killer cells and production if interleukin-12 and inhibition of NF-kappaB due to stimulation of TNF-α. All these regulated processes are responsible for anti-cancer effect of neem. Further research must be conducted to isolate and utilize other bioactive components that may be used for treatment of cancer.

Immunostimulant Effect

Immune system is composed of wide range of organs, tissues and highly complex cells that are involved in protecting human body from any harm. Immune system is responsible for providing immunity to healthy tissues against disease causing agents. Literature shows that neem leaves extract has immunostimulant activity because cell-mediated and humoral immunity was enhanced by aqueous neem leaves extract. Oral intake of neem leaves extract at a dose of 100 mg/kg body weight results in increase of antibodies including IgG and IgM (Ray *et al*., 1996).

Cell mediated immune response was enhanced due to activation of lymphocyte population and T-helper cells (TH-1) component. Phagocytic activity of macrophages was enhanced but alternative and classical C pathways were decreased after intake of neem leaves extract. Biological responses induced by tumor necrosis factor (TNF) were inhibited due to interaction of azadirachtin with binding domain and receptors of TNF (Thoh *et al*., 2010).

A study was done on infection caused in fish by *Vibrio harveyi* against lates calacrifier and Asian sea bass by using supplements prepared from neem leaves extract. Neem leaf extract was given at six different levels 0 g, 1 g, 2 g, 3 g, 4 g and 5 g/kg feed of fish. Results showed that neem leaves supplementation enhance activity of serum anti-protease, bactericidal activity, serum lysozyme, production of superoxide anion and enhanced activity of phagocytes. Survival rate and immune system of fish were improved after intake of neem supplementation (Talpur and Ikhwanuddin, 2013).

One of the volatile components from neem oil named as NIM-76 was isolated and its immunomodulatory properties were studied. Single intraperitonial injection of NIM-76 resulted in decreased lymphocytes counts and an increase in polymorphonuclear leukocytes (PMN). It was observed that this immunomodulatory effect is concentration dependent. Humoral component of immunity remained unaffected at a dose of 120 mg/kg while there was increase in lymphocytes proliferation and enhanced macrophage activity. Macrophage activity remained unaffected at a concentration of 300 mg/kg of NIM-76 while it stimulated the mitogen induced lymphocytes proliferation. This study concluded that NIM-76 activates lymphocytes and macrophages through cell mediated mechanisms (Sairam *et al*., 1997).

Effect of acetone water neem leaf extract on $CD4^+$ cells was investigated in 60 HIV/AIDS patients to determine the effect of neem on viral load in HIV/AIDS patients and on immunity. Patients were diagnosed as HIV positive having $CD4^+$ cell count less than 300 cells/mL. These patients were treated with 1 g acetone water neem leaf extract for 12 weeks. At the

end of the study trial, an increase in $CD4^+$ cells was observed. Mean body weight was increased up to 12%, hemoglobin concentration up to 24% and lymphocytes differential count was increased up to 20%. HIV/AIDS related pathogenesis was significantly reduced. Liver and kidney functions were not disturbed during this treatment. Results of this study support the safety of acetone water neem leaf extract against HIV/AIDS and its significant influence on $CD4^+$ cells due to which it can be used in preparation of multiple drugs combination therapies for HIV/AIDS patients (Mbah *et al*., 2007).

Neem is responsible for enhancing the immunity especially cell mediated and humoral immunity. It is responsible for increasing the antibody production especially IgG and IgM, activation of lymphocytes and macrophages through cell mediated mechanisms which are mainly attributed to the presence of several biologically active components in neem. Neem can be used in preparation of multiple drugs for boosting immunity especially in HIV/AIDS patients because of its significant influence on $CD4^+$ cells which are protective against HIV viruses.

Orodental Protection

Many teeth related problems can be cured by using neem plant. Orodental conditions such as perionditis and gingivitis can be cured by using neem leaves. Periodontal disorders including plaque oral infections can be inhibited and cured by using neem leaves. Bacterial content of *Streptococcus mutans* and *Streptococcus lactobacilli* as well as plaque can be decreased by using neem gel containing 25 mg/g of neem leaves extract (Pai *et al*., 2004).

Some new researches showed that oral health, perionditiis and type 2 diabetes mellitus are correlated with each other. For this purpose, antibacterial activity of neem is tested in the form of toothpaste prepared from neem or neem stick was used as a whole in both diabetic and normal individuals. Results of the study showed that oral micro biota were similar in healthy individuals as well as type 2 diabetes mellitus patients while bacterial load was high in diabetic patients which were significantly reduced after use of neem stick (Anbalagan *et al*., 2017).

Increased level of oral microbioata is mainly observed in many mouth related disorders such as perionditis and gingivitis. Microbial content in mouth is also increased in diabetic patients as well. Use of neem twing is an ancient civilization for cleaning teeth because it is responsible for eliminating several harmful bacteria in mouth, therefore, it must be used to clean teeth due to its orodental protection properties and preventing the diseases that are mainly associated through movement of these harmful bacteria from mouth to the gastrointestinal tract.

Skin Protection Effect

Long before medicinal drugs have been used to treat skin disorders but these drugs are failed to treat chronic skin conditions. Literature shows that neem has remarkable properties to treat chronic skin conditions. A study was performed on skin conditions including ringworm, scabies and eczema that can be cured by using lotion prepared from 70% alcoholic neem leaves extract (Singh *et al*., 1979). A study was performed to treat ringworm infection by using benzoic acid, salicylic acid and alcoholic neem leaves extract. Results of this study showed that neem leaves extract is more effective in treating ringworm infection as compared to salicylic and benzoic acid. Another study showed that scabies can be treated by using turmeric powder and fresh neem leaves in the ratio 1:4 (Charles and Charles, 1992).

Organic neem oil of high quality proved protective against chronic skin conditions including stubborn warts, ringworm, eczema, psoriasis and acne. Neem oil used as part of cosmetics to restore, beautify and clear the skin (Kumar and Navaratnam, 2013). Neem leaves are now extensively being used in many cosmetics and other products that are responsible for providing benefits against skin related infections as well as for glowing the skin. These products may include neem-based lotions, soaps and face wash. Neem oil is also being prepared by using its fruit and seeds that is mainly used to restore, beautify and clear the skin.

Ulcer Treating Effect

Ulcer is basically considered a lesion which if left untreated leads to chronic complications and even death of the person. Neem leaves show strong anti-ulcerogenic activity. *Helicobacter pylori* are major stomach ulcer causing bacteria. Stomach ulcer and inflammation caused by these bacteria can be treated by using neem leaves due to antibacterial and antihistamine compounds present in neem leaves (Febry *et al*., 1996).

Problems related to digestive system including ulcers can be cured traditionally due to medicinal and ayurvedic properties of neem. Neem is responsible in promotion of healthy digestive system due to its role in removal of harmful bacteria and toxins and stomach protection (Bandyopadhyay *et al*., 2004).

Another study was done in which gastric acid secreting volume, total and free acidity and ulcer index was reduced after neem leaves extract administration. Antiulcer properties of neem leaves are attributed due to their activity for degranulation of mast cells, prevention of mucous depletion, inhibition of H-K-ATPase that will block acid secretion, preventing apoptosis and oxidative damage. Nimbidin is potent anti-ulcer constituent of neem leaves that has anti-ulcer effect due to its antihistaminic properties (Chattopadhyay *et al*., 2004).

A study was done on albino rats in which gastric ulcer were induced by 40 mg/kg body weight of indomethacin. Neem leaves extract at a dose of 100-250 mg/kg body weight intraperitoneal and 100-800 mg/kg body weight per os show significant reduction in gastric ulcer. 100% cytoprotection was observed at 250 and 800 mg/kg body weight. Gastric ulcer was inhibited due to reduction in gastric acidity. For investigating the anti-ulcer mechanism of neem this study was conducted on gastric acid secretion by using 0.12 mg/kg body weight of cimetidine, 1 mg/kg body weight histamine, extract and histamine in combination and alone was administered to rats. Gastric acid secretion was significantly inhibited after intake of histamine and 250 mg/kg body weight of *Azadirachta indica* extract. Results showed that bark of neem contain antiulcer compounds through H_2 receptor and histamine (Raji *et al*., 2004).

Azadirachta indica (neem) bark aqueous extract has antiulcer and antisecretory properties. Acid secretion induced by drug was inhibited after administration of 2 mg/kg body weight of aqueous bark extract. Ligation of pylorus was also inhibited after intake of 2.7 mg/kg body weight of aqueous bark extract. Gastric ulcer induced by indomethacin was inhibited after intake of 1.25 mg/kg body weight of bark extract while gastric ulcer induced by resistant cold stress was also inhibited after administration of 1.5 mg/kg body weight of aqueous bark extract of neem. Aqueous bark extract was more effective in inhibiting acid secretion induced by pylorus ligation as compared to other drugs. Aqueous bark extract of neem has gastro protective activity due to inhibition of H-K-ATPase activity, depletion of endogenous glutathione and prevention of mucous adherence. Gastric mucosa oxidative damage was prevented by bark extract of neem because per oxidation of lipids was blocked as well as scavenging of endogenous hydroxyl radicals was done which is basically a major cause of ulcer (Bandyopadhyay *et al*., 2002).

Ulcer is basically a lesion which if left untreated will leads to chronic inflammation and ultimately cancer progression. Neem leaves contain some biologically active components that have strong anti-ulcerogenic properties. Neem is effective against ulcer because it inhibits the bacterial growth responsible for ulcer formation as well as it is effective against histamine. It can be used for the removal of harmful bacteria and toxins from the gastrointestinal tract and providing protection to the stomach. Most important component that has antihistamine properties is nimbidin. Some of the antioxidants present in neem leaves as well as in bark are responsible for maintaining the antioxidant status of the body because reactive free radicals are the major cause of ulcer formation.

Anti-pyretic, Anti-inflammatory and Neuropharmacological Effect

Pyrexia is defined as feverous condition in which body temperature rise above normal. Neem leaves show protective effect against fever. Traditionally, neem leaves are used to treat fever. Nimbidine is an active ingredient in neem leaves that show anti-pyretic properties. A study was done in which neem leaves extract showed anti-inflammatory effect at a dose of 400-800 mg/kg body weight with 75% ethanolic extract of neem leaves. Anti-analgesic and anti-pyretic effect was observed by using ethanolic neem leaves extract obtained from ether soluble fraction (Subapriya and Nagini, 2005). Epoxyazadiradione in as anti-inflammatory agent present in neem that show protective effect against macrophage migration inhibitory factor (Alam *et al*., 2012). Biological responses mediated by retinoic acid were inhibited by azadirachtin when it interacts with receptors of retinoic acid (Thoh *et al*., 2011).

Various activities of nervous system can be controlled by using neem leaves extract especially neem has been widely used against depression. A study showed that intake of neem leaves extract at a dose of 200 mg/kg body weight can be used to provide significant antidepressant activity (Jaiswal *et al*., 1994). Several bioactive components are present in neem that are responsible for multiple health effects such as nimbidine is responsible for reducing the fever and epoxyazadiradione has anti-inflammatory properties. Several bioactive components are also responsible for antidepressanrt properties due to which neem has neuropharmacological effect.

Anti-microbial Effect

Malaria is feverish condition commonly caused by malarial parasites. Literature showed that alcoholic extract of fresh neem leaves contain liminoids including nimbandiol, nimocinol, isomeldenin and meldenin that are effective against malaria fever caused by *Plasmodium falciparum* (Vasanth *et al*., 1990).

Many bacterial species are responsible for causing diseases. These bacterial species mainly include gram positive and gram-negative microorganisms such as Streptomycin and Mycobacterium tuberculosis. Growth of these microorganisms can be controlled by using neem leaves oil because it contains an active liminoid mahmoodin which has significant antibacterial activity (Siddique *et al*., 1992). Results of a study showed that neem oil contain an ester which has strong anti-bacterial activity (Zhong *et al*., 2010).

Fungi are responsible for causing many infections especially *Candida* species. Neem leaves extract has anti-fungal activity mainly due to presence of limonoid gedumin and volatile sulfides. A study was done on *Aspergillus parasiticus* infection and results of this study showed that aflatoxin activity was inhibited

after neem leaves extract administration (Allameh *et al*., 2002).

There are many viral diseases that can be cured by using neem leaves extract. Studies showed that aqueous neem leaves extract is effective against *measles* and *Vaccinia* virus. Results of a study showed that tender leaves with 10% water extract have strong anti-viral activity. Replication of dengue virus is also inhibited by administration if neem leaves extract. Another study showed that hepatitis B virus DNA polymerase was inhibited after neem leaves extract administration (Parida *et al*., 2002).

Another study was done on anti-viral activity of neem which showed that poliovirus was inhibited due to presence of polysaccharides present in neem (Galhardi *et al*., 2012). Polysaccharides were extracted from neem leaves by using water which showed protective effect against bovine herpes virus type-1 (BoHV-1) (Saha *et al*., 2010). Extracts of neem seed kernel showed protective effect against duck plague virus (Xu *et al*., 2012). Extract from bark of *Azadiracta indica* showed protective effect against infection caused by herpes simplex type- 1 virus (Tiwari *et al*., 2010).

Traditionally, neem is being used to clean the body from parasites because neem rapidly kills internal as well as external parasites. Neem extracts are responsible for anti-parasitic activity because they prevent the hatching of eggs of parasites, inhibit the feeding ability of parasites and interfere with life cycle of parasites because neem resemble to hormones for these actions (Kumar and Navaratnam, 2013).

Neem leaves contain different biologically active components that are responsible for anti-microbial activities. Various liminoids are present that are effective against malaria fever, some esters present in neem oil are responsible for anti-bacterial activity, aflatoxin activity after fungal infection can be inhibited by using neem leaves which shows that neem has anti-fungal activities. Neem is also effective against certain viruses responsible for causing different infectious diseases in humans as well as in plants. Neem resembles to hormones and interferes with the life cycle of parasites due to which it also has anti-parasitic properties.

Anti-hyperglycemic Activity

Diabetes mellitus is a group of metabolic disorders characterized by elevated blood glucose levels resulting due to defects in insulin action, insulin secretion or both. This disorder leads to abnormal metabolism of fats, proteins and carbohydrates. Insulin is hormone produced by beta cells of pancreas and required for proper use of body storage fuels (Mahan and Escott-Stump, 2013).

Insulin was isolated in 1922 by Best and Banting and a diabetic patient was treated by using this isolated insulin. Insulin is a peptide hormone having molecular weight 5808 Da and composed of 51 amino acids. Mammalian pancreas contains islets of Langerhans where β-cells are involved in synthesis of insulin. Pro-insulin precursor molecule is involved in synthesis of insulin in the presence of proteolytic enzymes such as exoprotease, carboxypeptidase E and prohormone convertase (PC1 and PC2). This enzymatic activity results in removal of C-peptide from insulin. α and β chains then combine together through disulphide linkage to form insulin (Booth *et al*., 2013).

In the body, fat and carbohydrate metabolism is mainly regulated by insulin. Energy nutrients present in blood includes fatty acids, glucose and amino acids are up taken by cells of body signaled by insulin after intake of meal. Insulin stimulates fat, glycogen and protein synthesis in muscles and liver in order to maintain normal blood glucose levels. In the absence of insulin, metabolism of energy yielding nutrients changes (Mahan and Escott-Stump, 2013).

Two types of diabetes mellitus have been reported including type 1 diabetes mellitus and type 2 diabetes mellitus. Type 1 diabetes mellitus is also known as insulin dependent diabetes mellitus (IDDM) or juvenile diabetes. It accounts for 5-10% of total diabetic cases. It is an autoimmune disorder in which beta-cells of pancreas are mistakenly destroyed by antibodies resulting in no insulin production and individual is at risk of ketoacidosis. Type 1 DM patients should receive exogenous insulin either in the form of injection or by using insulin pumps. Insulin should not be taken orally because it is a protein and can be destroyed by gastrointestinal enzymes (Nelms *et al*., 2010).

Most prevalent form of diabetes is type 2 diabetes mellitus accounting for 90-95% of diabetic cases. It mainly results due to decreased sensitivity of cells to insulin or insulin resistance. Glucose is continuously made by liver cells, but adipose and muscle cells are unable to remove glucose from blood. To overcome this situation pancreas secrete large amount of insulin resulting in hyperinsulinemia. Hyperglycemia worsens with the passage of time when pancreas becomes less able to overcome this situation. Beta cells are exhausted due to high demand for insulin leading to impaired insulin secretion and carbohydrate metabolism. Type 2 DM risk can be increased by physical inactivity, aging, smoking, poor dietary habits, increase alcohol consumption and abdominal obesity (Whitney and Rolfes, 2016).

To decrease blood glucose level before the identification of insulin, neem leaves extract was useful to control hyperglycemia. A research was performed on streptozotocin induced diabetic rabbits in which

effect of neem leaves extract was studied on hepatic glycogen storage and intravenous glucose tolerance test before and after administration of neem leaves extract. Epinephrine action was mimicked after administration of neem leaves extract on peripheral utilization of blood glucose level (Chattopadhyay, 1996).

A study was performed in which inhibitory role of serotonin on release of insulin is blocked by ingestion of neem leaves extract (Chattopadhyay, 1999). Petroleum ether extract of neem seeds (2 g/kg body weight) and neem leaves extract (0.9 g/kg body weight) was given to streptozotocin induced diabetic rats for 28 days. This treatment resulted in significant decrease in serum lipid peroxidase (LPO), catalase (CAT), superoxide dismutase (SOD) and serum glucose levels (Gupta *et al*., 2004).

A study was performed by using combined extract of *Azadirachta indica* (neem) and *Vernonia amygdaline* (African bitter leaf) on diabetes and oxidative stress. 25 diabetic mice were separated into five groups and treated with chlorpropamide, bitter leaf only, neem only, saline, combined extract of neem and bitter leaf for 24 days. Blood glucose level was monitored after oral administration of 4.28 mg/kg body weight of chlorpropamide and 400 mg/kg body weight of extract of each leaf. After 7-hour administration of neem leaf extract, reduction in blood glucose level was 28.56%, for chlorpropamide reduction was 60.51%, after bitter leaf extract administration reduction was 47.31% and after combined extract administration reduction was 24.78%. Results showed that neem leaves extract provide best results against blood glucose level and liver abnormalities including GOT and GPT (Ebong *et al*., 2008).

A study was performed on four important medicinal plants including *Ocimum sanctum*, *Azadirachta indica*, *Gymnema sylvestre* and *Catharanthus roseus* on streptozotocin-induced diabetic and normal rats and blood glucose levels were estimated. As compared to other plant leaves extract, *Azadirachta indica* leaves extract showed significant reduction in blood glucose level (Chattopadhyay, 1999).

Phytochemicals such as liminoids and azadiradione in neem leaves act as strong inhibitors of human pancreatic alpha-amylase thus lowering post-parandial glucose level. These liminoids bind and inactivate alpha-amylase and are considered as basic anti-diabetic target to control blood glucose level (Ponnusamy *et al*., 2015).

For induction of diabetes mellitus, streptozotocin was injected in male wistar rats at a dose of 55 mg/kg body weight through tail bone. Effect of neem seed husk (NSH) at a dose of 0.9 mg/kg body weight and neem seed kernel (NSK) at a dose of 2 mg/kg body weight was administered for 28 days. Significant reduction was observed in serum creatine phosphokinase (CPK) and lipid per oxidation (LPO) after neem seed, neem kernel and insulin administration. Serum catalase (CAT) and superoxide dismutase (SOD) was normalized after neem seed kernel (NSK) and neem seed husk (NSH) administration. Cardiac damage was protected after NSH, NSK and insulin administration. Oxidative stress in erythrocytes and heart of STZ induced diabetic rats was decreased after NSH and NSK administration (Gupta *et al*., 2004).

Diabetes mellitus is the most prevalent chronic metabolic disorder that can be prevented or treated by using an ancient ethno medicinal plant, neem. Active components in neem are responsible for modulating the several metabolic pathways in the body responsible for diabetes. Liminoids present in neem leaves are the major anti-diabetic agents because they are responsible for inhibiting the key carbohydrate metabolizing enzyme including alpha-amylase and alpha-glucosidase. Oxidative stress can also be reduced by using neem leaves which is the major cause as well as complication of diabetes mellitus.

Antioxidant Activity

With the passage of time, role of free radicals is increasing in progression of many health-related disorders including cardiovascular diseases, cancer, rheumatoid arthritis, inflammation and diabetes (Sithisarn *et al*., 2005). During metabolism inside the human body natural by-products are produced that may be harmful to human health and are known as free radicals. Free radicals are basically electrically charged molecules that interact with multiple enzymes present in human body, altering the normal function of proteins, negatively interact with nucleic acids, damage cell membranes and cells also. Cells lose their functions and structure due to presence of these free radicals which is known as oxidative stress (Govindarajan *et al*., 2005).

Literature showed that hypoglycemia may be a causative agent for one of the diabetic complications known as oxidative stress. For diabetic patients, main sources of free radicals are elevated glycosylation of proteins related to hyperglycemia and glucose itself. In diabetes, increased glucose level results in slow but significant non-enzymatic proteins glycosylation. Diabetes incidence mainly involves the presence of hydroxyl radicals and peroxide anion (Baynes and Thorpe, 1999).

Diabetogenic action can be prevented by some hydroxyl radical scavengers such as dimethyl urea, ethanol, serum catalase (CAT) and superoxide dismutase (SOD). Toxic electrophiles can be neutralized by glutathione (GSH) which is provided by enzymes such as glutathione-S-transferase (GST) and glutathione reductase (GSH-R). Studies showed that

free radicals are responsible for injury of tissues in diabetic patients. Oxidative stress may also be caused due to decreased level of defense system based on antioxidants, elevated oxidation of lipids and increased plasma lipids level (Govindarajan *et al*., 2005).

Bark and leaf extract of *Azadiracta indica* has highest antioxidant activity (Ghimeray *et al.*, 2009). A study demonstrated that strong antioxidant activity is present in bark, stem, flowers and leaves of Siamese neem tree (Sithisarn et al., 2005). Activity of antioxidants in *Azadiracta indica* leaves was determined by using various extracts including butanol, hexane and chloroform. Best results were obtained from chloroform extract (Hossain *et al*., 2013).

To estimate the antioxidant activity of neem seed oil and flowers, ethanolic extract at a concentration of 200 ug/ml was used. Results indicated that seeds and flowers had greatest radical scavenging activity (Nahak and Sahu, 2011). Stem, bark, fruits, leaves and flowers were studied for antioxidant activities and results showed greatest free radical scavenging activity with 50% scavenging activity at 30.6 ug/ml of ethanolic bark extract, 27.9 ug/ml of ethanolic flower extract and 26.5 ug/ml of aqueous ethanolic leaf extract. Total antioxidant activity of leaves was 0.959 mM, flowers were 0.988 mM and of bark was 1.064 mM (Sithisarn *et al*., 2005).

In another study, oxidative stress was induced in male wistar rats by using N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and protective effect of ethanolic extract of neem leaves was investigated against oxidative stress. Three different concentrations of neem leaves extract including 400 mg/kg body weight, 200 mg/kg body weight and 100 mg/kg body weight were given for five consecutive days and levels of different antioxidant enzymes including GST, GPx, GSH, CAT and SOD were determined. Elevated lipid per oxidation was observed in erythrocytes and after treatment with ethanolic extract of neem leaves, levels of oxidative stress biomarkers were reduced significantly (Subapriya *et al.*, 2003).

A study was performed on hepatic stress by using methanolic extract of *Azadirachta indica* leaves. 500 mg/kg body weight of methanolic extract of neem leaves was given to rats for five days. After treatment with neem leaves hepatic stress parameters were estimated including apoptosis markers by using real time PCR, tissue changes and antioxidants levels in the tissues. Levels of antioxidant enzymes such as SOD, CAT, GPx, GST and glutathione were increased (Dkhil *et al*., 2013).

A study was done on male Swiss albino rats for evaluating the effect of pretreatment with ethanolic extract of neem leaves against MNNG induced oxidative stress and genotoxicity. Rats were divided into four groups. Rats of group 1 received 40 mg/kg body weight of MNNG through intragastric incubation. Rats of group 2 received 200 mg/kg body weight of ethanolic extract of neem leaves for five days through intragstric feeding followed by administration of MNNG 1.5 h after final feeding. Rats in group 3 received only ethanolic extract of neem leaves for five days. Rats in group 4 were used as control group by using similar volume of normal saline. Results of this study showed that MNG-induced lipid per oxidation was decreased and antioxidant status was increased in red blood cells, liver and stomach after pretreatment with ethanolic extract of neem leaves (Subapriya *et al*., 2004).

A study was done in relation to antioxidant activity of neem tree by using its flowers on animal modeling. Results of the study showed that there was elevated level of phase II antioxidant enzymes such as glutathione-S-transferase (GST) and reduced activity of phase I enzymes including aminopyrone-N-demethylase (AMD), aniline hydroxylase (ANH) and cytochrome P450 (Kusamran *et al*., 1998). In another study, induction of quinine reductase activity was observed by using chloroform extract of flowers from neem tree (Kuakulkiat, 2001).

Oxidative stress and nephrotoxicity was induced in rats by injecting cisplatin (CP) at a dose of 5 mg/kg body weight. Methanolic extract of neem leaves was given at a dose of 500 mg/kg body weight through gastric gavage for 5 days. Results of study showed decreased apoptosis in renal tissues, decreased expression of nuclear factor kappa B, increased activities of antioxidant enzymes including glutathione peroxidase, glutathione-S-transferase (GST), glutathione reductase, catalase (CAT), superoxide dismutase (SOD) and elevated levels of glutathione. Administration of neem leaves extract also normalized the production of nitric oxide (NO), malondialdehyde (MDA), decreased serum creatinine, urea and uric acid. Histological studies showed that administration of neem leaves extract protect kidneys from oxidative damage. Down regulation of genes including Bax, caspase-9 and caspase-3 was also observed in animal models treated with methanolic extract of neem leaves (Moneim *et al*., 2014).

Oxidative stress in the body is mainly caused due to formation of reactive oxygen and nitrogen species. Neem leaves are rich in antioxidants that can be used to prevent this oxidative stress by decreasing the peroxidation of lipids and improving the antioxidant status of the body. Neem flowers are also responsible for ameliorating the oxidative stress by inducing quinine reductase activity. Neem is also responsible for down regulating the several genes involved in oxidative stress.

Anti-Hyperlipidemic Potential

Hyperlipedemia or dyslipidemia is referred to abnormal lipid profile of the blood which is also induced due to abnormal blood glucose concentrations. A study was performed to elucidate the effect of neem leaves extract on cardiovascular diseases induced by hyperglycemia. Results of the study showed that levels of HDL remain unchanged while a significant decrease in serum overall lipids, triglycerides, VLDL, LDL and total cholesterol was observed after administration of neem leaves extract as compared to diabetic control animals (Chattopadhyay and Bandyopadhyay, 2005).

Azadirachta indica, a medicinal plant has been studied from ancient times for its therapeutic effect on serum lipid profile. A study was conducted to elucidate the therapeutic potential of neem leaves extract against hyperglycemia and hyperlipidemia. Rats were divided into four groups: first group was control group, second was diabetic control, third group was diabetic group received alcoholic extract of neem leaves and the fourth group was diabetic group received glibencalamide as a standard. Results of the study showed that administration of ethanolic extract of neem leaves normalized the blood glucose and lipid levels. This study showed that ethanolic extract of *Azadirachta indica* leaves reverses the dyslipidemia (Bisht and Sisodia, 2010).

In Indian ayurvedic medicinal system, neem leaves are being used to treat diabetes and to normalize the lipid levels as well. In a recent study, an active component named azadiractolide which is basically a tetranorterpenoid was isolated from neem leaves and its effect on serum lipid and glucose level was determined in STZ-induced diabetic rats. Azadiractolide was administered at a dose of 50 and 100 mg/kg body weight to STZ-induced diabetic rats intraperitoneally once a week for one month. Results of the study showed significant decrease in fasting blood glucose, total cholesterol, low density lipoprotein and very low density lipoprotein while there was appreciable increase in high density lipoprotein level. Azadiractolide administration also showed appreciable inhibitory effects on alpha glucosidase (IC50 value of 47.85 ±1.4 µg/ ml) and alpha amylase (IC50 value of 55.80 ± 1.7 µg/ml). Thus, azadiractolide can be used as a potential component for management of dyslipidemia (Kumar *et al*., 2011).

Literature revealed that neem leaves extract is also beneficial in managing hypertension. A study was performed in which rats were divided into three groups: first group was control group receiving soybean oil along with normal drinking water, second group was DOCA-Salt group receiving 15 mg/kg of DOCA salt containing 1% NaCl and 0.03% KCl along with normal drinking water and the third group was DOCA-Salt-neem group receiving DOCA salt along with 20 mg/kg/day aqueous neem leaves extract. Blood pressure was measured weekly, and results revealed that mean arterial blood pressure was significantly decreased in control group (97 ± 3.7 mmHg) as well as in DOCA salt neem group (87 ± 3.4 mm Hg) as compared to DOCA salt group (115 ± 7.1 mm Hg). This study proved that administration of 20 mg/kg/day neem leaves extract along with DOCA salt results in reduction of hypertension (Obiefuna and Young, 2005).

Literatue showed that neem is also effective in reversing the dyslipidemia by decreasing the level of triglycerides, low density lipoprotein and very low-density lipoprotein but increasing the level of high-density lipoprotein. Main component in neem leaves responsible for managing dyslipidemia is azadiractolide. It is also responsible for decreasing the symptoms of hypertension.

Hepatoprotective Activity

Several studies have been performed regarding hepatoprotective activity of neem leaves extract against hepatic injury induced by using paracetamol. Results of a study showed that administration of neem leaves extract to rats having paracetamol hepatotoxicity reverses the levels of TBARS, sodium-potassium ATPase activity, liver and blood glutathione. Microsomal enzymes are involved in metabolism of paracetamol that results in formation of free radicals. Neem leaves extract act as free radical scavenger by trapping reactive oxygen species and preventing the per oxidation of lipids. *Azadirachta indica* enhances the level of blood and liver GSH (glutathione peroxidase) that protects the tissues against oxidative stress. Over the many bioactive components present in neem leaves, rutin and quercetin were mainly observed for their hepatoprotective activity (Chattopadhyay, 2003).

Hepatotoxicity in rats was induced by using paracetamol at a dose of 2 g/kg and effect of aqueous leaf extract of neem was observed on disturbed lipid parameters. Administration of 500 mg/kg body weight of neem leaves extract resulted in decreased levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glutamyl transpeptidase (γ-GT). Histological and macroscopical studies showed decrease necrosis in liver along with decreasing the level of liver major enzymes (Bhanwra *et al.*, 2000).

Hepatoprotective activity of young stem bark extract of neem was determined by inducing hepatic damage through carbon tetrachloride. Bark extract was given to rats at a dose of 200 and 500 mg/kg. Results of the study showed that administration of *Azadirachta indica* bark extract stabilize the levels of SGOT/AST, SGPT/ALT, ALP and bilirubin. It also elevates the level of total proteins. Thus, this plant can be used for improving the functional status of liver cells (Gomase *et al.*, 2011).

Another study was reported in which hepatocytes of rats were used by dividing the isolated hepatocytes into five groups. First group of hepatocytes was given the normal solution, second group was receiving 10 µL of carbon tetrachloride (CCl_4), third group was receiving CCl_4 and aqueous extract of neem leaves at a dose of 10, 50 and 100 µg/mL, fourth group was given CCl_4 and methanolic extract of neem leaves at a dose of 10, 50 and 100 µg/mL and the fifth group was receiving CCl_4 along with 15, 30 and µg/mL of silymarin. CCl_4 results in hepatotoxicity due to its metabolite CCl_3 that act as a free radical result in per oxidation of lipids by binding with lipoproteins. *Azadirachta indica* has hepatoprotective activity because it preserves the normal hepatic physiological mechanisms. Hepatoprotective activity was determined by measuring the levels of AST, ALP GSH and GPT. Results of the study showed that levels of these enzymes are reduced in groups of hepatocytes receiving aqueous and methanolic extract of neem leaves as compared to groups receiving only CCl_4 (Devmurari and Jivani, 2010).

Another research was conducted in which hepatotoxicity in rats was induced by using CCl_4 and the effect of one of the active constituents of neem named as azadirachtin-A was investigated. Wistar rats were divided into four groups: first group was control group, second group was treatment group receiving 1 ml/kg of CCl_4, third group was receiving 100 µg/kg of silymarin along with CCl_4 and the fourth group was receiving different concentrations of azadirachtiA including 100 and 200 µg/kg/day along with CCl_4. Study duration was nine days after which blood was collected for serum biochemical analysis and for pathological examination liver tissue was obtained. Results of the study showed that CCl_4 was responsible for causing liver damage and decreasing albumin and total protein levels and a significant rise in serum levels of ALP, ALT, AST and BUN was recorded. Liver toxicity caused by CCl_4 can be prevented by dose dependent pretreatment with azadirachtin-A which results in reduced hepatocellular necrosis and protecting the liver against damage and restoring the liver to normal (Baligar *et al*., 2014).

Neem is responsible for improving the liver function because of the presence of several bioactive components. Hepatoprotective activity is mainly attributed to the presence of rutin and quercitin that are responsible for decreasing the necrosis in liver cells. Another bioactive component present in neem leaves is azadirachtin-A that results in decreasing necrosis in hepatic cells and ultimately restoring the liver functions to normal.

Renal Protection

Renal toxicity is also referred as nephrotoxicity and it is mainly caused due to chemical substances that are affecting the kidneys functions. Literature revealed that neem has protective effect against nephrotoxicity. To prove this statement several studies have been performed on cisplatin-induced renal damage in wistar rats. One of the researches was conducted in which rats were divided into three groups. Group one was control group, group two was given 10 mg/kg intraperitonial injection of cisplatin, group three was treated with 500 mg/kg/day of neem leaves extract along with 10 mg/kg of cisplatin for 14 days. Serum creatinine, urea and electrolytes were determined after collecting blood through cardiac puncture. Results showed that neem leaves extract pre-treatment normalized the levels of serum creatinine, urea and electrolytes as well as reversed the kidney apoptosis and necrosis. Therefore, it can be concluded that neem leaves extract has potential to attenuate nephrotoxicity and protecting the kidney functions in rats (Kpela *et al*., 2012).

A comparative study of effect of vitamin E and neem leaves extract was performed on cisplatin induced renal damage in rats. Rats were divided into four groups: Group I was control group, group II was given 10 mg/kg of cisplatin through intraperitonial injection, group III was receiving 10 mg/kg of cisplatin injection intraperitonially along with 500 mg/kg/day oral administration of neem leaves extract for 14 days, group IV was receiving 10 mg/kg intraperitonial injection of cisplatin along with oral administration 6 mg/kg/day of vitamin E for 14 days. At the end of the study, blood was collected for biochemical analysis through cardiac puncture and serum creatinine, urea and electrolytes level were determined. Kidneys were removed and stained by using deoxyribonucleic acid (DNA) and hematoxylin and eosin (H and E) dyes. Results showed that elevated serum creatine, urea and electrolytes as well as tubule cells necrosis was normalized in the group receiving neem leaves extract as compared to group receiving vitamin E. Therefore, it was concluded that neem leaves extract has potential to attenuate nephrotoxicity in wistar rats irrespective to the vitamin E (Kpela *et al*., 2013).

Cisplatin was responsible for causing nephro and hepatotoxicity in rats. A study was conducted to demonstrate the effect of neem leaves extract against this cisplatin induced hepatic and nephrotoxicity. Neem leaves extract showed significant protection against hepatic and renal damage by normalizing the levels of serum urea, uric acid, creatinine, total bilirubin, ALP, AST, ALT and γ-GT. Neem leaves extract has this protective potential against histopathological injury induced by cisplatin. This study concluded that pre, co and post treatment with neem leaves extract can protect the liver and kidneys against cisplatin induced toxicity (Ezz-Din *et al*., 2011).

Xenobiotics are one of the major major causative agents of hepatic and renal damage. In modern era, there is no specific medicine for the

treatment of these disorders due to which medicinal herbs have been used since ancient times to tackle these disorders. A study was carried out in which sodium benzoate was administered sub chronically to induce hepatic and renal damage and protective effects of neem leaves were observed. Rats were divided into four groups namely A, B, C and D. group A received distilled water, group B was given 200 mg/kg of sodium benzoate, group C was given 200 mg/kg sodium benzoate along with 200 mg/kg *Azadirachta indica* leaf extract and group D was given 200 mg/kg sodium benzoate along with 500 mg/kg *Azadirachta indica* extract. Results of the study suggested that sodium benzoate hepatic and renal toxicity was ameliorated after administration of neem leaves extract that normalized the levels of serum creatine, uric acid, urea, alaninine aminotransferase (ALT) and aspartate aminotransferase (AST) (Oyewole *et al*., 2012).

Cisplatin is one of the anti-neoplastic agents but it is toxic to kidney and liver but this cisplatin induced hepatic and renal toxicity can be mediated by using neem leaves extract. Renal and hepatic histochemical staining showed weak protein and polysaccharide staining in cisplatin treated group. Group treated with pre, post and co treatment by using methanolic extract of neem leaves showed strong staining for proteins and polysaccharides as compared to control group.

These findings suggest that depletion of polysaccharides in liver and kidney due to cisplatin can be reverted after administration of neem leaves extract (Soliman *et al*., 2013).

Besides providing other health benefits, neem is also protective against nephrotoxicity and improving the renal function. Toxicity mainly results in decreasing the level of several essential nutrients including polysaccharides, but these levels can be restored after neem leaves extract administration and normalizing the levels of serum urea, creatinine, uric acid and electrolytes those results in improving the renal function.

Effect on hematological indices

Hematological parameters are affected by neem intake that may include hemoglobin, PCV, RBC, MCV, MCH, MCHC, WBC, lymphocytes and neutrophil. Neem leaves can be used as a component of livestock feed in Nigeria because of presence of several bioactive components in neem leaves that has effect on hematological parameters. The biologically active components mainly include nimbidin, nimbin, salanin, meliantriol and azadirachtin. A study was conducted to investigate the effect of neem leaves-based diet on blood blood chemistry. Experimental units were rabbits divided into four groups. Rabbits in group 1 served as control treated with 0% neem leaf meal (NLM) whereas group 2 was given 5% NLM, group 3 was treated with 10% NLM and group 4 was given 15% NLM. Results of the study demonstrated that neutrophil count in group 2, 3 and 4 was significantly reduced. Neem leaf meal administration significantly decreased the serum ALP and cholesterol. As compared to control group, serum glucose and globulin concentrations were also reduced in group 4. These results indicated that neem leaf meal-based diets have significant effect on hematological indices (Ogbuewu *et al*., 2010).

Ethno medicinal plants including neem have been used since ancient times as a component of feed ingredient for many animals. A study was conducted on crossbred New Zealand white typed rabbit bucks to explore the effect of neem leaf meal on serum biochemistry of these rabbit bucks. For this purpose, rabbits were into four groups and are treated with four different treatments: rabbits received T_1 with 0% neem leaf meal, T_2 received 5% neem leaf meal, T_3 received 10% neem leaf meal and T_4 received 15% neem leaf meal. Results of study showed that serum globulin level was significantly reduced in T_2 and T_3 as compared to T4. Serum sodium levels were significantly different in T_2 and T_4 as compared to T_1. Serum urea level was significantly high in T_3 and T_4 as compared to T_1 and T_2. ALP level was significantly affected in T_2 and T_3. Serum glucose and serum cholesterol synthesis was significantly reduced in groups receiving neem leaf-based treatment as compared to control group which showed that neem leaf has potential to attenuate serum biomarkers (Ogbuewu *et al*., 2010).

A study was carried out to evaluate the effect of aqueous extract of *Azadirachta indica* leaves (ALE) on serum biochemistry, hematology and growth of broiler chicks. Four different types of treatments were used including T_1 receiving 0 mL ALE, T_2 receiving 20 mL ALE, T_3 receiving 40 mL ALE and T_4 receiving 60 mL ALE. Water and feed were provided ad libitum. No significant difference was observed regarding protein efficiency ratio, feed conversion, water and feed intake as well as weight gain. There was significant influence of ALE on serum hematological parameters including packed cell volume, hemoglobin, red blood cells, white blood cells, MCV, MCH and MCHC. ALE also had significant effect on serum total protein, albumin, globulin, urea, creatinine, glucose and cholesterol levels. This research concluded that ALE can be used for production of broiler chicks without any harmful effects on blood parameters (Nnenna and Okey, 2013).

One of the most important medicinal plants is *Azadirachta indica* because it is rich source of many alkaloids including salanin azadirachtin and nimbitin that contribute to the general medicinal properties of the plant. A study was designed to explore the effect of *Azadirachta indica* (AI) consumption on serum glucose level as well as hematological parameters during pregnancy and lactation. For this purpose, female rats were divided into three groups: lactating, pregnant and

non-pregnant rats. Depending on the dose of AI administration, each group is subdivided into four subgroups. Subgroup I received distilled water because it served as control group throughout research. Subgroup II received 200 mg/kg, subgroup III received 400 mg/kg and subgroup IV received 600 mg/kg of AI for 21 days. At the end of the study duration, blood sample was collected for estimation of blood glucose and hematological parameters after overnight fasting. There was significant dose dependent decrease in blood glucose level and a significant increase in platelet (PLT), white blood cells (WBCs), red blood cells (RBCs), hemoglobin concentration (Hb) and packed cell volume (PCV). From this research, it was concluded that AI can be used as hematopoietic agent in ameliorating the burden of hyperglycemia and anemia in women during pregnancy (Iyare and Obaji, 2014).

A study was conducted to evaluate the protective effect of methanolic fraction (MF) and ethyl acetate fraction (EAF) from crude ethanolic extract (CEE) of *Azadirachta indica* (Neem) leaves against various free radicals and hydrogen peroxide (H_2O_2) induced damage to pBR322 DNA and red blood cells (RBCs). Neem leaves fractions reduced free radicals such as nitric oxide (NO), hydroxyl ($OH^{\cdot}$), superoxide ($O^{\cdot-}$), $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ to their non-radical form. These neem leaves fractions significantly mitigate H_2O_2 induced oxidative damage to pBR322 DNA and RBCs. This protective effect is due to presence of protective compounds such as quercetin and nimbolide. Results of this study suggested that protective and antioxidant effects of active neem leave fractions against H_2O_2 induced lipid per oxidation and Pbr322 DNA damage is mainly due to their ability to inhibit various free radicals (Manikandan *et al*., 2009).

A 28 day study trial was conducted on starter broiler to evaluate the effect of *Azadirachta indica* (Neem) leaf meal on serum biochemical and hematological parameters. Neem leaves were harvested manually, air dried and milled to form neem leaf meal. Broilers were divided into five different groups receiving five different types of diet: group I was receiving 0%, group II was receiving 2.5%, group III was receiving 5%, group IV was receiving 7.5% and group V was receiving 10% neem leaf meal. At the end of the study trial, blood was collected and analyzed for biochemical and hematological indices. Results of the study showed that packed cell volume (PCV) and hemoglobin (Hb) levels were not reduced significantly. Basophills, eosinophills and monocytes traces were not observed. Serum cholesterol level was significantly reduced due to inclusion of neem leaf meal, but serum glucose level was raised. There was decrease in serum levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphates (ALP). Results also showed that cation/anion exchange boosting capacity and integrity of kidneys was also maintained even at 10% level of neem leaf meal that maintain the level of serum electrolytes including bicarbonates, chloride, potassium, sodium and calcium. This study concluded that there was no harmful effect of neem leaf meal on physiological parameters (Obikaonu *et al*., 2011).

Effect of dietary neem leaf meal (NLM) on blood chemistry, linear body measurements and body weight gain was investigated through a 16-week feeding trial on pre-pruberbal buck rabbits. Four treatments of NLM were prepared including 0% that act as control, 5%, 10% and 15% NLM. At the end of study trial, it was observed that lymphocytes count of rabbits fed on control diet was higher than rabbits fed on 15% NLM. Mean cell volume (MCV) and mean cell hemoglobin (MCH) was significantly higher in control group as compared to group fed on 5% NLM. Neutrophill count of group fed 15% NLM was significantly different as compared to other groups. Results of this study suggested that NLM can be tolerated up to 15% without any harmful effects on hematological parameters, linear body measurements and body weight gain (Ogbuewu *et al*., 2010).

Neem is rich source of many alkaloids including salanin, azadirachtin and nimbitin. These components have hematopoietic properties due to which they can be used for ameliorating anemia in pregnant women. Several other components are present in neem leaves that are responsible for improving the hemoglobin level and increasing the synthesis of red blood cells.

CONCLUSION

Ethno medicinal plants should be taken into consideration in order to prevent and treat lifestyle related disorders as well as to maintain health. Due to increasing cost of modern medicine, people are now moving towards the use of natural plants as well as their products to treat ailments. *Azadirachta indica* (Neem) is one of the most important traditional medicinal plants that have been used since centuries due to presence of many biologically active components that are effective against cancer, abnormal lipid level, elevated sugar level, liver damage, renal toxicity and many others. Due to these reasons, this important medicinal plant should be used in modern medicinal system to assist the population. Further research is required for isolation, purification and characterization of many biologically active components in different parts of this plant. These components should be used for prevention and treatment of different health related disorders and for preparation of several functional and nutraceutical foods for human consumption.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

REFERENCES

- Abdel Moneim, A. E., Othman, M. S., & Aref, A. M. (2014). Azadirachta indica attenuates cisplatin-induced nephrotoxicity and oxidative stress. *BioMed research international*, *2014*.
- Akudugu, J., Gäde, G., & Böhm, L. (2001). Cytotoxicity of azadirachtin A in human glioblastoma cell lines. *Life sciences*, *68*(10), 1153-1160.
- Alam, A., Haldar, S., Thulasiram, H. V., Kumar, R., Goyal, M., Iqbal, M. S., ... & Bandyopadhyay, U. (2012). Novel anti-inflammatory activity of epoxyazadiradione against macrophage migration inhibitory factor: inhibition of tautomerase and proinflammatory activities of macrophage migration inhibitory factor. *Journal of Biological Chemistry*, *287*(29), 24844-24861.
- Allameh, A., Abyaneh, M. R., Shams, M. R., Rezaee, M. B., & Jaimand, K. (2002). Effects of neem leaf extract on production of aflatoxins and activities of fatty acid synthetase, isocitrate dehydrogenase and glutathione S-transferase in Aspergillus parasiticus. *Mycopathologia*, *154*(2), 79-84.
- Anbalagan, R., Srikanth, P., Mani, M., Barani, R., Seshadri, K. G., & Janarthanan, R. (2017). Next generation sequencing of oral microbiota in Type 2 diabetes mellitus prior to and after neem stick usage and correlation with serum monocyte chemoattractant-1. *Diabetes Research and Clinical Practice*, *130*, 204-210.
- Arivazhagan, S., Balasenthil, S., & Nagini, S. (1999). Neem leaves extract against cancer. Med. Sci. Res. 20, 27-37.
- Babu, P. V. A., Liu, D., & Gilbert, E. R. (2013). Recent advances in understanding the anti-diabetic actions of dietary flavonoids. *The Journal of nutritional biochemistry*, *24*(11), 1777-1789.
- Bailey, C. J., & Day, C. (1989). Traditional plant medicines as treatments for diabetes. *Diabetes care*, *12*(8), 553-564.
- Baligar, N. S., Aladakatti, R. H., Ahmed, M., & Hiremath, M. B. (2014). Hepatoprotective activity of the neem-based constituent azadirachtin-A in carbon tetrachloride intoxicated Wistar rats. *Canadian journal of physiology and pharmacology*, *92*(4), 267-277.
- Bandyopadhyay, U., Biswas, K., Chatterjee, R., Bandyopadhyay, D., Chattopadhyay, I., Ganguly, C. K., ... & Banerjee, R. K. (2002). Gastroprotective effect of Neem (Azadirachta indica) bark extract: Possible involvement of H+-K+-ATPase inhibition and scavenging of hydroxyl radical. *Life sciences*, *71*(24), 2845-2865.
- Bandyopadhyay, U., Biswas, K., Sengupta, A., Moitra, P., Dutta, P., Sarkar, D., ... & Banerjee, R. K. (2004). Clinical studies on the effect of Neem (Azadirachta indica) bark extract on gastric secretion and gastroduodenal ulcer. *Life sciences*, *75*(24), 2867-2878.
- Baral, R., & Chattopadhyay, U. (2004). Neem (Azadirachta indica) leaf mediated immune activation causes prophylactic growth inhibition of murine Ehrlich carcinoma and B16 melanoma. *International immunopharmacology*, *4*(3), 355-366.
- Barik, S., Bhuniya, A., Banerjee, S., Das, A., Sarkar, M., Paul, T., ... & Baral, R. (2013). Neem leaf glycoprotein is superior than Cisplatin and Sunitinib malate in restricting melanoma growth by normalization of tumor microenvironment. *International immunopharmacology*, *17*(1), 42-49.
- Baynes, J. W., & Thorpe, S. R. (1999). Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes*, *48*(1), 1-9.
- Bhanwra, S., Singh, J., & Khosla, P. (2000). Effect of Azadirachta indica (Neem) leaf aqueous extract on paracetamol-induced liver damage in rats. *Indian journal of physiology and pharmacology*, *44*(1), 64-68.
- Bisht, S., & Sisodia, S. S. (2010). Anti-hyperglycemic and antidyslipidemic potential of Azadirachta indica leaf extract in STZ-induced diabetes mellitus. *Journal of Pharmaceutical Sciences and Research*, *2*(10), 622.
- Booth, A. O., Lowis, C., Dean, M., Hunter, S. J., & McKinley, M. C. (2013). Diet and physical activity in the self-management of type 2 diabetes: barriers and facilitators identified by patients and health professionals. *Primary health care research & development*, *14*(3), 293-306.
- Bose, A., & Baral, R. (2007). Natural killer cell mediated cytotoxicity of tumor cells initiated by neem leaf preparation is associated with CD40–CD40L–mediated endogenous production of interleukin-12. *Human immunology*, *68*(10), 823-831.
- Cardellina, J. H. (2002). Challenges and opportunities confronting the botanical dietary supplement industry. *Journal of Natural Products*, *65*(7), 1073-1084.
- Charles, V., & Charles, S. X. (1992). The use and efficacy of Azadirachta indica ADR ('Neem') and Curcuma longa ('Turmeric') in scabies. A pilot study. *Tropical and Geographical Medicine*, *44*(1-2), 178-181.
- Chattopadhyay, I., Nandi, B., Chatterjee, R., Biswas, K., Bandyopadhyay, U., & Banerjee, R. K. (2004). Mechanism of antiulcer effect of Neem (Azadirachta indica) leaf extract: effect on H+-K+-ATPase, oxidative damage and apoptosis. *Inflammopharmacology*, *12*(2), 153-176.
- Chattopadhyay, R. (2003). Possible mechanism of hepatoprotective activity of Azadirachta indica leaf

extract: part II. *Journal of ethnopharmacology*, *89*(2-3), 217-219.

- Chattopadhyay, R. R., & Bandyopadhyay, M. (2005). Effect of Azadirachta indica leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. *African Journal of Biomedical Research*, *8*(2), 101-104.
- Chattopadhyay, R.R. (1996). Possible mechanism of antihyperglycemic effect of *Azadirachta indica* leaf extract: *Part IV. J Gen Pharmacol. 27*, 431-434.
- Chattopadhyay, R.R. (1999). Possible mechanism of antihyperglycemic effect of *Azadirachta indica* leaf extract: Part V. *J Ethnopharmacol, 67*, 373-376.
- Dasgupta, T., Banerjee, S., & Yadava, P.K. (2004). Anti-cancer effect of neem against stomach and skin cancer. J. *Ethnopharmacol, 12*, 92-98.
- Devmurari, V. P., & Jivani, N. P. (2010). Hepatoprotective activity of methanolic and aqueous extracts of Azadirchata indica leaves. *International Journal of PharmTech Research*, *2*(2), 1037-1040.
- Dkhil, M.A., Al-Quraishy, S., Aref, A.M., Othman, M.S., El-Deib, K.M., & Moneim, A.E.A. (2013). The potential role of *Azadirachta indica* treatment on cisplatin-induced hepatotoxicity and oxidative stress in female rats. *J Oxid Med Cell Longev. 13*, 1-9.
- Ebong, P. E., Atangwho, I. J., Eyong, E. U., & Egbung, G. E. (2008). The antidiabetic efficacy of combined extracts from two continental plants: Azadirachta indica (A. Juss)(Neem) and Vernonia amygdalina (Del.)(African bitter leaf). *American journal of Biochemistry and Biotechnology*, *4*(3), 239-244.
- Elumalai, P., Gunadharini, D. N., Senthilkumar, K., Banudevi, S., Arunkumar, R., Benson, C. S., ... & Arunakaran, J. (2012). Induction of apoptosis in human breast cancer cells by nimbolide through extrinsic and intrinsic pathway. *Toxicology letters*, *215*(2), 131-142.
- Elumalai, P., Gunadharini, D. N., Senthilkumar, K., Banudevi, S., Arunkumar, R., Benson, C. S., ... & Arunakaran, J. (2012). Ethanolic neem (Azadirachta indica A. Juss) leaf extract induces apoptosis and inhibits the IGF signaling pathway in breast cancer cell lines. *Biomedicine & Preventive Nutrition*, *2*(1), 59-68.
- Ezz-Din, D., Gabry, M. S., Farrag, A. R. H., & Moneim, A. E. A. (2011). Physiological and histological impact of Azadirachta indica (neem) leaves extract in a rat model of cisplatin-induced hepato and nephrotoxicity. *Journal of Medicinal Plants Research*, *5*(23), 5499-5506.
- Fabry, W., Okemo, P., & Ansorg, R. (1996). Activity of East African medicinal plants against Helicobacter pylori. *Chemotherapy*, *42*(5), 315-317.
- Faccin-Galhardi, L. C., Yamamoto, K. A., Ray, S., Ray, B., Linhares, R. E. C., & Nozawa, C. (2012). The in vitro antiviral property of Azadirachta indica polysaccharides for poliovirus. *Journal of Ethnopharmacology*, *142*(1), 86-90.
- Ghimeray, A. K., Jin, C. W., Ghimire, B. K., & Cho, D. H. (2009). Antioxidant activity and quantitative estimation of azadirachtin and nimbin in Azadirachta indica A. Juss grown in foothills of Nepal. *African Journal of Biotechnology*, *8*(13).
- Gomase, P. V., Rangari, V. D., & Verma, P. R. (2011). Phytochemical evaluation and hepatoprotective activity of fresh juice of young stem (tender) bark of Azadirachta indica A. Juss. *International Journal of Pharmacy and Pharmaceutical Sciences*, *3*(2), 55-59.
- Govindarajan, R., Vijayakumar, M., & Pushpangadan, P. (2005). Antioxidant approach to disease management and the role of 'Rasayana'herbs of Ayurveda. *Journal of ethnopharmacology*, *99*(2), 165-178.
- Gupta, S., Kataria, M., Gupta, P. K., Murganandan, S., & Yashroy, R. C. (2004). Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. *Journal of Ethnopharmacology*, *90*(2-3), 185-189.
- Hossain, M. A., Al-Toubi, W. A., Weli, A. M., Al-Riyami, Q. A., & Al-Sabahi, J. N. (2013). Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. *Journal of Taibah University for Science*, *7*(4), 181-188.
- Humber, J.M. (2002). The role of complementary and alternative medicine: Accommodating pluralism. JAMA. 288, 1655-1656.
- Ibrahim, K. S., & El-Sayed, E. M. (2016). Potential role of nutrients on immunity. *International Food Research Journal*, *23*(2).
- Iyare, E. E., & Obaji, N. N. (2014). Effects of aqueous leaf extract of Azadirachta indica on some haematological parameters and blood glucose level in female rats. *Nigerian Journal of Experimental and Clinical Biosciences*, *2*(1), 54.
- Jaiswal, A. K., Bhattacharya, S. K., & Acharya, S. B. (1994). Anxiolytic activity of Azadirachta indica leaf extract in rats. *Indian journal of experimental biology*, *32*(7), 489-491.
- Kpela, T., Ekanem, T. B., & Akpantah, A. O. (2012). Protective effect of ethanol extract of neem leaves on cisplatin-induced kidney damage in wistar rats. *Journal of Medical Sciences*, *1*(9), 249-254.
- Kpela, T., Umoren, E. B., & Andrew, A. D. (2013). Comparative study of the protective effects of ethanol extract of neem leaves and vitamin E on cisplatin-induced kidney damage in Wistar rats. *Journal of Medicine and Medical Sciences*, *2*(5), 114-119.

- Kuakulkiat, W. (2001). *Cancer chemopreventive components from Azadirachta indica A. Juss flowers*. Mahidol University.
- Kumar, D., Mitra, A., & Manjunatha, M. (2011). Azadirachtolide: An anti-diabetic and hypolipidemic effects from Azadirachta indica leaves. *Pharmacogn. Commun*, *1*, 78-84.
- Kumar, V. S., & Navaratnam, V. (2013). Neem (Azadirachta indica): Prehistory to contemporary medicinal uses to humankind. *Asian Pacific journal of tropical biomedicine*, *3*(7), 505-514.
- Kusamran, W. R., Ratanavila, A., & Tepsuwan, A. (1998). Effects of neem flowers, Thai and Chinese bitter gourd fruits and sweet basil leaves on hepatic monooxygenases and glutathione S-transferase activities, and in vitro metabolic activation of chemical carcinogens in rats. *Food and Chemical Toxicology*, *36*(6), 475-484.
- Lamson, D. W., & Brignall, M. S. (2000). Antioxidants and cancer, part 3: quercetin. *Alternative medicine review: a journal of clinical therapeutic*, *5*(3), 196-208.
- Mahan, L.K., & Escott-Stump, S. (2013). Krause's Food and Nutrition Therapy. 12th ed. Saunders, Elseiver: Canada.
- Mahapatra, S., Karnes, R. J., Holmes, M. W., Young, C. Y., Cheville, J. C., Kohli, M., ... & Donkena, K. V. (2011). Novel molecular targets of Azadirachta indica associated with inhibition of tumor growth in prostate cancer. *The AAPS journal*, *13*(3), 365-377.
- Manikandan, P., Letchoumy, P. V., Gopalakrishnan, M., & Nagini, S. (2008). Evaluation of Azadirachta indica leaf fractions for in vitro antioxidant potential and in vivo modulation of biomarkers of chemoprevention in the hamster buccal pouch carcinogenesis model. *Food and Chemical Toxicology*, *46*(7), 2332-2343.
- Mbah, A. U., Udeinya, I. J., Shu, E. N., Chijioke, C. P., Nubila, T., Udeinya, F., ... & Obioma, M. S. (2007). Fractionated neem leaf extract is safe and increases CD4+ cell levels in HIV/AIDS patients. *American journal of therapeutics*, *14*(4), 369-374.
- Nahak, G., & Sahu, R. K. (2011). Evaluation of antioxidant activity of flower and seed oil of Azadirachta indica A. juss. *Journal of Applied and Natural Science*, *3*(1), 78-81.
- Nanduri, S., Thunuguntla, S. S. R., Nyavanandi, V. K., Kasu, S., Kumar, P. M., Ram, P. S., ... & Venkateswarlu, A. (2003). Biological investigation and structure–activity relationship studies on azadirone from Azadirachta indica A. Juss. *Bioorganic & medicinal chemistry letters*, *13*(22), 4111-4115.
- Nelms, M., & Sucher, K. P. (2015). Nutrition therapy and pathophysiology.
- Nnenna, O. P., & Okey, A. A. (2013). Toxicity and nutritional assessment of aqueous Azadirachta indica (neem) leaf extract in broiler chicks. *International Journal of Biosciences*, *3*(6), 172-180.
- Obiefuna, I., & Young, R. (2005). Concurrent administration of aqueous Azadirachta indica (neem) leaf extract with DOCA-salt prevents the development of hypertension and accompanying electrocardiogram changes in the rat. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *19*(9), 792-795.
- Obikaonu, H. O., Okoli, I. C., Opara, M. N., Okoro, V. M. O., Ogbuewu, I. P., Etuk, E. B., & Udedibie, A. B. I. (2011). Haematological and serum biochemical indices of starter broilers fed neem (Azadirachta indica) leaf meal. *Online Journal of Animal and Feed Research*, *1*(4), 150-154.
- Ogbuewu, I. P., Okoli, I. C., & Iloeje, M. U. (2010). Evaluation of toxicological effects of leaf meal of an ethnomedicinal plant-neem on blood chemistry of puberal chinchilla rabbit does. *Report and Opinion*, *2*(2), 29-34.
- Ogbuewu, I. P., Uchegbu, M. C., Okoli, I. C., & Iloeje, M. U. (2010). Toxicological effects of leaf meal ethnomedicinal plant—neem—on serum biochemistry of crossbred New Zealand white typed rabbit bucks. *Rep Opin*, *2*(2), 54-57.
- Ogbuewu, I. P., Uchegbu, M. C., Okoli, I. C., & Iloeje, M. U. (2010). Assessment of blood chemistry, weight gain and linear body measurements of pre-puberal buck rabbits fed different levels of Neem (Azadirachta indica A. Juss.) leaf meals. *Chilean Journal of Agricultural Research*, *70*(3), 515-520.
- Oyewole, O. I., Dere, F. A., & Okoro, O. E. (2012). Sodium benzoate mediated hepatorenal toxicity in wistar rat: Modulatory effects of azadirachta indica (neem) leaf. *European Journal of Medicinal Plants*, *2*(1), 11.
- Pai, M. R., Acharya, L. D., & Udupa, N. (2004). Evaluation of antiplaque activity of Azadirachta indica leaf extract gel—a 6-week clinical study. *Journal of ethnopharmacology*, *90*(1), 99-103.
- Parida, M. M., Upadhyay, C., Pandya, G., & Jana, A. M. (2002). Inhibitory potential of neem (Azadirachta indica Juss) leaves on dengue virus type-2 replication. *Journal of ethnopharmacology*, *79*(2), 273-278.
- Patel, V.K., & Venkatakrishna, B.H. (1998). Effect of neem in the treatment of oral infections and plaque growth inhibition in periodontal disorders. Int. J. Clin. Pharmacol. Ther. Toxicol. 26, 176-180.
- Patwardhan, B., Warude, D., Pushpangadan, P., & Bhatt, N. (2005). Ayurveda and traditional Chinese medicine: a comparative overview. *Evidence-based*

complementary and alternative medicine, *2*(4), 465-473.

- Ponnusamy, S., Haldar, S., Mulani, F., Zinjarde, S., Thulasiram, H., & Ravikumar, A. (2015). Gedunin and azadiradione: Human pancreatic alpha-amylase inhibiting limonoids from neem (*Azadirachta indica*) as anti-diabetic agents. J Appl Nat Sci. 10, 1-19.
- Puri, H. S. (1999). *Neem: the divine tree Azadirachta indica*. CRC Press.
- Raji, Y., Ogunwande, I. A., Osadebe, C. A., & John, G. (2004). Effects of Azadirachta indica extract on gastric ulceration and acid secretion in rats. *Journal of ethnopharmacology*, *90*(1), 167-170.
- Ray, A., Banerjee, B. D., & Sen, P. (1996). Modulation of humoral and cell-mediated immune responses by Azadirachta indica (Neem) in mice. *Indian journal of experimental biology*, *34*, 698-701.
- Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free radical biology and medicine*, *20*(7), 933-956.
- Saha, S., Galhardi, L. C., Yamamoto, K. A., Linhares, R. E. C., Bandyopadhyay, S. S., Sinha, S., ... & Ray, B. (2010). Water-extracted polysaccharides from Azadirachta indica leaves: Structural features, chemical modification and anti-bovine herpesvirus type 1 (BoHV-1) activity. *International Journal of Biological Macromolecules*, *47*(5), 640-645.
- SaiRam, M., Sharma, S. K., Ilavazhagan, G., Kumar, D., & Selvamurthy, W. (1997). Immunomodulatory effects of NIM-76, a volatile fraction from Neem oil. *Journal of ethnopharmacology*, *55*(2), 133-139.
- Scambia, G., Mancuso, S., Panici, P. B., De Vincenzo, R., Ferrandina, G., Bonanno, G., ... & Capelli, A. (1993). Quercetin induces type-II estrogen-binding sites in estrogen-receptor-negative (MDA-MB231) and estrogen-receptor-positive (MCF-7) human breast-cancer cell lines. *International journal of cancer*, *54*(3), 462-466.
- Schumacher, M., Cerella, C., Reuter, S., Dicato, M., & Diederich, M. (2011). Anti-inflammatory, pro-apoptotic, and anti-proliferative effects of a methanolic neem (Azadirachta indica) leaf extract are mediated via modulation of the nuclear factor-κB pathway. *Genes & nutrition*, *6*(2), 149-160.
- Siddiqui, S., Faizi, S., & Siddiqui, B. S. (1992). Constituents of Azadirachta indica: isolation and structure elucidation of a new antibacterial tetranortriterpenoid, mahmoodin, and a new protolimonoid, naheedin. *Journal of Natural Products*, *55*(3), 303-310.
- Singh, N., Misra, N., Singh, S. P., & Kohli, R. P. (1979). Melia azadirachta in some common skin disorders-a clinical evaluation. *Antiseptic*, *76*, 677-679.
- Sithisarn, P., Supabphol, R., & Gritsanapan, W. (2005). Antioxidant activity of Siamese neem tree (VP1209). *Journal of Ethnopharmacology*, *99*(1), 109-112.
- Soliman, D. E. E. I., Gabry, M. S., Farrag, A. R. H., & Moniem, A. E. A. (2013). The Protective Role of Neem Leaves Extract on Cisplatin-Induced Polysaccharides and Protein Depletion in Rat Liver and Kidney. *Pakistan Journal of Zoology*, *45*(6).
- Srivastava, P., Yadav, N., Lella, R., Schneider, A., Jones, A., Marlowe, T., ... & Chandra, D. (2012). Neem oil limonoids induces p53-independent apoptosis and autophagy. *Carcinogenesis*, *33*(11), 2199-2207.
- Subapriya, R., & Nagini, S. (2005). Medicinal properties of neem leaves: a review. *Current Medicinal Chemistry-Anti-Cancer Agents*, *5*(2), 149-156.
- Subapriya, R., Bhuvaneswari, V., & Ramesh, V. (2004). Neem tree for solving global problems. Cell Biochem Funct, 15, 22-32.
- Subapriya, R., Kumaraguruparan, R., Abraham, S. K., & Nagini, S. (2005). Protective effects of ethanolic neem leaf extract on N-methyl-N′-nitro-N-nitrosoguanidine-induced genotoxicity and oxidative stress in mice. *Drug and chemical toxicology*, *27*(1), 15-26.
- Subapriya, R., Kumaraguruparan, R., Chandramohan, K.V.P., & Nagini, S. (2003). Chemo protective effects of ethanolic extract of neem leaf against MNNG induced oxidative stress. *J Pharmazie*, *58*, 512-517.
- Talpur, A. D., & Ikhwanuddin, M. (2013). Azadirachta indica (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, Lates calcarifer challenged with Vibrio harveyi. *Fish & shellfish immunology*, *34*(1), 254-264.
- Talpur, A. D., & Ikhwanuddin, M. (2013). Azadirachta indica (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, Lates calcarifer challenged with Vibrio harveyi. *Fish & shellfish immunology*, *34*(1), 254-264.
- Thoh, M., Babajan, B., Raghavendra, P. B., Sureshkumar, C., & Manna, S. K. (2011). Azadirachtin interacts with retinoic acid receptors and inhibits retinoic acid-mediated biological responses. *Journal of Biological Chemistry*, *286*(6), 4690-4702.
- Thoh, M., Kumar, P., Nagarajaram, H. A., & Manna, S. K. (2010). Azadirachtin interacts with the tumor necrosis factor (TNF) binding domain of its receptors and inhibits TNF-induced biological responses. *Journal of Biological Chemistry*, *285*(8), 13556.

- Tiwari, V., Darmani, N. A., Yue, B. Y., & Shukla, D. (2010). In vitro antiviral activity of neem (Azardirachta indica L.) bark extract against herpes simplex virus type-1 infection. *Phytotherapy Research*, *24*(8), 1132-1140.
- Vasanth, S., Gopal, R.H., & Rao, R.B. (1990). Plant anti-malarial agents. J. Sci. Ind. Res. 49, 68-77.
- Veeraraghavan, J., Natarajan, M., Lagisetty, P., Awasthi, V., Herman, T. S., & Aravindan, N. (2011). Impact of curcumin, raspberry extract, and neem leaf extract on rel protein-regulated cell death/radiosensitization in pancreatic cancer cells. *Pancreas*, *40*(7), 1107-1119.
- Villaseñor, I. M., & Lamadrid, M. R. A. (2006). Comparative anti-hyperglycemic potentials of medicinal plants. *Journal of Ethnopharmacology*, *104*(1-2), 129-131.
- Vinothini, G., Manikandan, P., Anandan, R., & Nagini, S. (2009). Chemoprevention of rat mammary carcinogenesis by Azadirachta indica leaf fractions: modulation of hormone status, xenobiotic-metabolizing enzymes, oxidative stress, cell proliferation and apoptosis. *Food and Chemical Toxicology*, *47*(8), 1852-1863.
- Whitney, E., & Rolfes, S.R. (2016). Understanding Nutrition. 14th Ed. Cenage Learning, Stamford: USA.
- World Health Organization (WHO). (2000). Report of the inter-regional workshop on intellectual property rights in the context of traditional medicine. Bangkok, Thailand. Available at: http://apps.who.int/medicinedocs/pdf/h2944e/h2944e.pdf. Accessed on October 10, 2019.
- Xu, J., Song, X., & Yin, Z.Q. (2012). Antiviral activity and mode of action of extracts from need seed kernel against duck plague virus in vitro. *Poult Sci*, *91*, 2802- 2807.
- Zhong, P., Yu, Q., & Zhong, Y. (2010). Antibacterial activity of 9-octadecanoic acid-hexadecanoic acidtetrahydrofuran-3,4-diyl ester from neem oil. *Agr. Sci. China*, *9*, 1236-1240.