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An overview of Neem (*Azadirachta indica*) and its potential impact on health

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ABSTRACT

Global health and medical practice seek to merge alternative medicine with evidence-based medicine for a better understanding of the metabolic process and its effects in the human body. An example is the use of complementary medicine like phytotherapy. Azadirachta indica (Neem), a tree originally from India and Myanmar, called by many "The village pharmacy" or "Divine tree" because of its many health properties. In recent times, Neem-derived extracts have been shown to work from anywhere from insect repellent, to supplements to lower inflammation, diabetic control, and even to combat cancer. Herein, we state the health benefits found in diverse compounds and extracts derived from Neem, highlighting the mechanisms and pathways in which Neem compounds produce their effects, while warning that the improper and unstandardized conditions to produce extracts can lead to health issues, particularly certain compounds might have damaging effects on the liver and kidneys.

1. Introduction

The World Health Organization refers to "Good-Health" as a state of physical and mental well-being not altered by any disease or ailment (Arumugam et al., 2014). Ancient Sanskrit had a particular expression for this state: "*Nimba*" (Sitasiwi, Isdadiyanto, & Mardiati, 2018), which over time, derived into Neem. Nowadays, Neem is used to reference the *Azadirachta indica* (Neem) tree, traditionally though to bring "good health" to those who take them (Arumugam et al., 2014; Omóbòwálé et al., 2016; Patel, Venkata, Bhattacharyya, Sethi, & Bishayee, 2016). Through this review we aim to highlight the latest work done on the extracts of Neem, focusing on certain major aspects, such as their importance as antioxidants, and their potential role in mitigating diabetes and cancer. Beforehand we will give a brief overview on several of the most relevant bioactive compounds typically found in many extracts, although though our work, we will continue referring to other compounds as it is understandable that both staring materials and extraction processes differ greatly. Furthermore, we emphasize that much of the current work continues to be experimental and as such, there is a section devoted to the toxicity effects, which should always be considered, encouraging further research to develop better products for human use. Finally, we discuss a section on industrial applications, as exemplified by it use as antimicrobial and fungicide agents, its effects as a contraceptive, derivatives for epoxy-resins, and other current medical use.

The Neem tree, is primarily cultivated in the southern regions of Asia and Africa, where it has been seen used through many ages, in medical folklore. We should note that various parts of the Neem tree, including the leaves, bark, fruit, flowers, oil, and gum are associated with the aforementioned medical folklore in the treatment of certain medical conditions such as cancer, hypertension, heart diseases, and diabetes. The potential effects that are seen when using these extracts can certainly be attributed cellular and molecular mechanisms, these mechanisms include free radical scavenging, detoxification, DNA repair, cell cycle alteration, programmed cell death mitigation and autophagy, immune surveillance, anti-inflammatory, anti-angiogenic, and anti-metastatic activities and the ability to modulate of various signaling pathways(Arumugam et al., 2014; Omóbòwálé et al., 2016; Patel et al., 2016).

Estimates of alternative medicine use today as primary care, are in the order of 80% for developing countries (Rupani & Chavez, 2018), while in developed (or industrialized) countries, the use of alternative medicine continues to gain popularity as a complementary way of care. An effect mostly attributed to migration; as more people move towards

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developed countries, they bring not only their skills, but their traditions and way of life (Deng et al., 2013). Hence, in places like India, Pakistan, and other eastern developing countries, we see practice of complementary alongside allopathic medicine, where several healing traditions standout such as Ayurveda and Sowa-Rigpa, as these traditions take root in balance and energy or a spiritual healing process. Notably, these traditions embark on the use of several therapies using a complex of herbs and plants, like Turmeric, Amla, Tuls, Guggul and Neem (Rupani & Chavez, 2018; Verma, Ponan, & Kamin, 2019). Interestingly, these mixtures nowadays represent the basis for many commercial products used in cosmetics, soaps, toothpaste, and pest repellents. In addition, by tradition they also continue as treatments for chickenpox, fever, headache, leprosy, jaundice, constipation, respiratory problems, rheumatism, and gastrointestinal disorders (Eid, Jaradat, & Elmarzugi, 2017; Heyman et al., 2017; Joshi, Bhat, Kothiwale, Tirmale, & Bhargava, 2010; Saleem, Muhammad, Hussain, & Bukhari, 2018). Over time, these proposed complexes of herbs and plants have been in more detail studied. Results have found that many of these herbs and plants contain several compounds mainly of the following families: flavonoids, catechins, anthocyanins, quercetins, saponins, tannins, limonoids, gallic acid and other minor polyphenols (Fig. 1); all known to have biological effects (Alzohairy, 2016; Heyman et al., 2017; Nagini, 2014).

Furthermore, traditional use has shown that benefits exist when consuming Neem (Al Akeel, Mateen, Janardhan, & Gupta, 2017; Ghonmode et al., 2013; Yerima et al., 2012). Therefore, the interest of different communities and researchers, to several parts of the Neem to produce extracts. Out of all, the oil appears to be the most widely used portion (Deng et al., 2013; Patel et al., 2016). Whether using oil-based or other extracts, a few drawbacks continue to arise. As, the lack of current information regarding toxicity levels and full characterization of compounds in not yet fully derived. Now, given that neem preparations have been consumed through different generations of people, it is fair to extrapolate that Neem-derived products are safe. To understand, we must inspect, the current state-of-the art on the basic techniques of extraction the known bioactive compounds, and methods of application, as these components should be key better understanding toxicity.

2. Methods

This review focuses on an overview of the current literature on Neem an its extracts, highlighting the importance of the compounds found via several extraction methods and from different parts of the plant. In addition, we also show through, how the different extracts are currently being explored for their potential benefit in human research, much of what is described refers to either animal model or *in-vitro* research. In certain cases, extracts have been used as alternative medicine and as such these will be mentioned, as well as derived products currently used. There are also 2 important aspects we considered the firs was the addition of toxicological studies as it is important to grasp both sides as in certain extracts or at certain dosages these can have negative effects.

For the development of this literature review, we conducted searches using both scientific databases e.g., PUBMED, Science Direct, and Elsevier for scientific studies, as well as, commercial search engines such as google, googlepatent and patenscope to search for commercial and patentable applications. For current research-based literature we used terms and bolean operators: “Neem” AND “Traditional medicine” OR “Alternative medicine” AND/OR “Bioactive compounds” OR “Chemical compounds”, and “Neem” AND “Antioxidants” AND/OR “Diabetes” AND/OR “Cancer” AND/OR “Inflammation”. For commercial applications we used terms such as “Neem-derived products”, or “Neem” AND patents”, or “Neem industrial applications”.

After careful consideration of the literature obtained, we took only those that would fit within the scope of our working review and proceeded to develop our database, and the production of this review

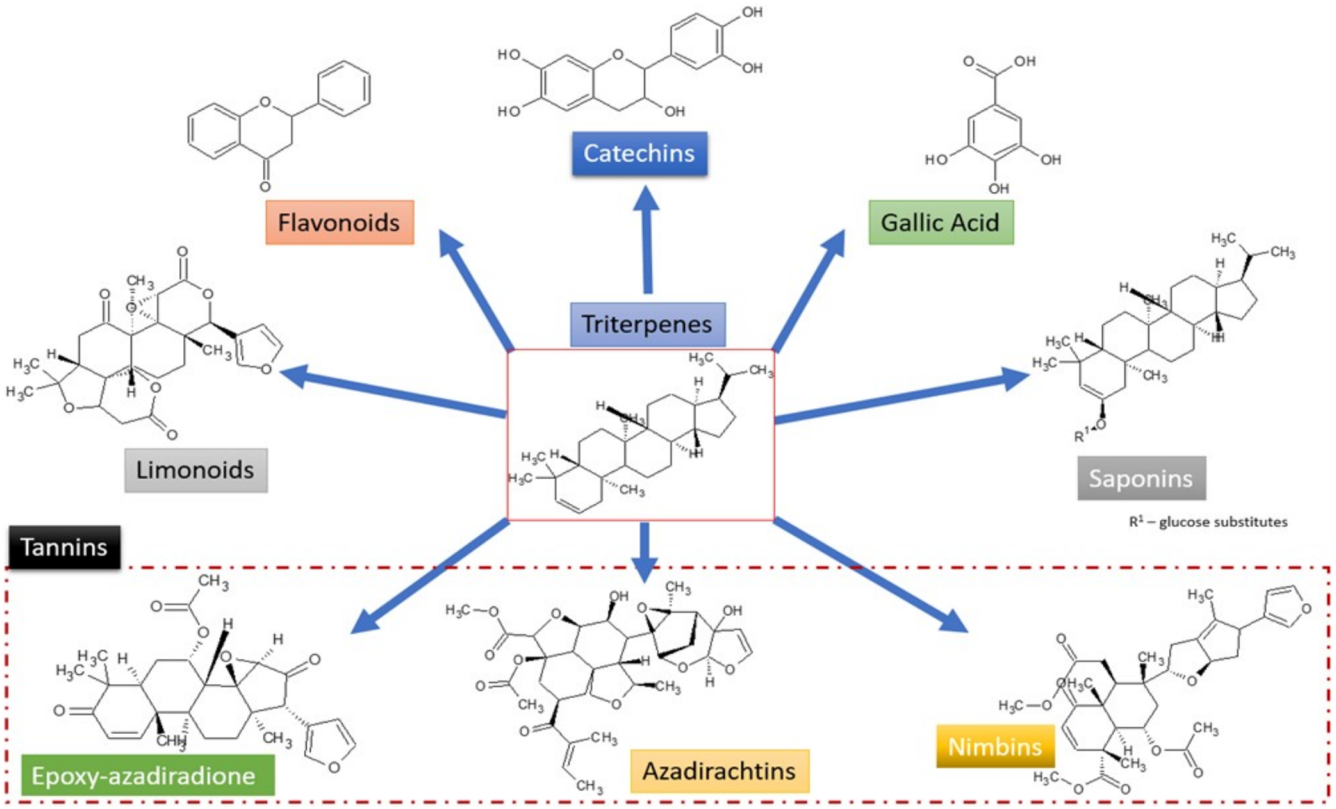

Fig. 1. (A) Types of extracts and mayor compounds found in Neem. (B) Examples of regulation. Neem compounds produce effects over important cellular mechanisms at a molecular level.

(Diagram 1).

3. Bioactive compounds present in *Azadirachta indica*

Over time, research has shown that Azadirachta indica is rich in a wide range of compounds, of which several have pharmacological potential. Out of all these compounds, triterpenes lead the way in having therapeutic use. In particular, Nimbin (triterpene) has shown to have antipyretic, fungicidal, antihistamine and antiseptic properties. Also Nimbin is associated with anti-inflammatory and antioxidant effects, therefore reducing damage by mitigating the production of reactive oxygen species. (Naik et al., 2014; Schumacher, Cerella, Reuter, Dicato, & Diederich, 2011). Also found in Neem are Flavonoids, which function as inhibitors of prostag-landin biosynthesis, and endoperoxides and the enzymes like protein kinases and phosphodiesterases, all involved in inflammation (Batista, Lima, Abrante, de Araújo, Batista, Abrante, & Magalhães, 2018; Hernández-Aquino & Muriel, 2018; Naik et al., 2014). As mentioned, oil extracts are the most typical used form of Neem and its in-depth phytochemical analysis has confirmed the presence in high amounts of triterpenes, flavonoids and saponins, while other components such as catechins and nimbins, seem to be present in lower amounts (Naik et al., 2014; Schumacher et al., 2011). Other metabolites found in Neem extracts are: limonoids, tannins, alkaloids, terpenoids, reducing sugar, catechins, sterols and gallic acid (Naik et al., 2014; Roma et al., 2015; Saleem et al., 2018; Schumacher et al., 2011).

The leaf of the Neem tree appears to have developed a particular set of glycoproteins named as neem leaf glycoprotein (NLGP) that when tested on mammalian subjects, showed immune-modulatory activity, providing the potential to restrict tumor growth by modulating local and systemic immunity (Banerjee et al., 2014; Dayakar, Chandrasekaran, Veronica, Sundar, & Maurya, 2015; Durrani et al., 2008; Kundu et al., 2018). Recently, Dash, Dixit, and Sahoo (2017) conducted an analysis involving leaf extracts (aqueous and methanoic indicating high levels of saponins, tannins and glycosides in the aqueous extracts. While methanoic extracts showed top levels of alkaloids, tannins, and flavonoids (Dash et al., 2017). Previous studies (non-methanoic) reported glycosides nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbioland in leaf extracts (Alzohairy, 2016), which shows the high variety of coumpounds available, but interestingly place much stress on the extraction process. Biochemical analysis done on leaf extracts has revealed high presence of proline, which is a current treatment for neurodegenerative diseases like Alzheimer's and Parkinson's disease, Type 2 Diabetes Mellitus and Polycythemia (Dash et al., 2017; Gladkevich et al., 2007; Mesgari-abbasi, Valizadeh, & Mirzakhani, 2019; Yenkoyan, Fereshetyan, Matinyan, Chavushyan, & Aghajanov, 2018).

Other recent studies by Hossaiń s group was able to characterize, leaf extracts, by up to 5 different extraction methods: hexane, ethyl-acetate, chloroform, butanol, methanol, and test for their antioxidant capacity, given that all these solvent have different polarities, each one showed interesting differences. Most importantly, chloroform extracts were deemed as having the highest antioxidant effect, mostly containing (2E)-3,7,11,15-tetramethyl- 2-hexadecen-1-ol, methyl 14-methylpentadecanoate, lineoleoyl chloride, phytol, methyl isoheptadecanoate and nonacosane. On the other side of the spectrum, the methanolic extracts show the lowest antioxidant effect. These extracts mainly contained: m-Toluylaldehyde, methyl 14-methylpentadecanoate, Lineoleoyl chloride, Methyl isoheptadecanoate. We should point out that the hexane-derived extract had the highest biologically active compounds: (2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Methyl petroselinate, Phytol, Methyl isoheptadecanoate, Hexadecamethylcyclooctasiloxane, Butyl palmitate, 2,6,10,14-Tetramethylheptadecane, Nonadecane, Isobutyl Stearate, Oxalic acid, 2-ethylhexyl tetradecyl est, Heptacosane, Eicosane, 7-hexyl- Heptacosane, 7-hexyl, and Octacosane. This same group, also determined gallic acid equivalents, as means of quantifying total phenolic compounds. They determined th–at butanol had the highest total phenolics (107.3 GA/g) and hexane had the lowest concentration (20.8 GA/g). Next, they quantified total flavonoids by UV over dry samples. They found the highest concentration of total flavonoids to be in the methanol extract (529.5 mg/100 g) and the lowest was in the butanol extract (63.0 mg//100 g) (Al-Hashemi & Hossain, 2016; Hossain, Al-Toubi, Weli, Al-Riyami, & Al-Sabahi, 2013; Khamis Al-Jadidi & Hossain, 2015).

Interestingly, methanolic extraction from the flowers have shown prenylated flavonoids (5,7,4′-trihydroxy- 8-prenylflavanone, 5,4′-dihydroxy-7-methoxy-8-prenylflavanone, 5,7,4′-trihydroxy-3′,8-diprenylflavanone, and 5,7,4′-trihydroxy-3′,5′- diprenylflavanone). Compounds determined not to be in the leaves, and which showed to have antimutagenic activity against Trp-P-I, Trp-P-II, and PhIP. As one might expect, flowers show a diverse variety of compounds such as flowerine, flowerone, O- methylazadironolide and diepoxyazadirol. Other known constituents present in flowers are triterpenoid (trichilenone acetate), flavanones, nimbaflavone, 3′-prenylnaringenin and 4-(2-hydroxyethyl) phenol (Saleem et al., 2018).

Neem-derived extracts have shown to play a role as antimicrobial and insecticide agents. A main constituent of Neem, Azadirachtin is a complex tetranortriterpenoid limonoid present in seeds, is accountable for the toxic effects in insects. Experiments have shown that ethanol extract of neem leaves showed in vitro antibacterial activity against both *Staphylococcus aureus* and MRSA (Al Akeel et al., 2017; Farjana, Zerin, & Kabir, 2014; Gupta, Ansari, Gupta, & Narwani, 2019; Quelemes et al., 2015). Although the antibacterial, antimicrobial and insecticide properties of Neem are not the focus of this review, we will discuss them in brief as commercial aspects of Neem.

4. Antioxidant effect

Free radicals or reactive oxygen species (ROS) are a major source of inflammation, as they act upon many biological molecules, exerting damage by taking out electrons as a way of entering a stable state, unleashing in the cell a state of oxidative stress (Alzohairy, 2016; Kiranmai, Mahender Kumar, & Ibrahim, 2011). Therefore, there is a need for providing adequate compounds (termed antioxidants) to stabilize or neutralize these radicals as a step in preventing or blocking an exacerbation of oxidative stress, which can lead to many diseases. These antioxidant molecules will supplement the work of the body's natural antioxidant defenses: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione (GSH), nitric oxide dioxygenase (NOD) (Basir & Shailey, 2012; Gautam, Gangwar, Singh, & Goel, 2015). To provide the body such compounds, a simple way is to supplement them in the diet. One way is to supplement with natural extracts like those derived from Neem; in forms such as teas and oils, seem to be a simple and cost-effective way to introduce antioxidants (Alzohairy, 2016; Farjana et al., 2014; Khamis Al-Jadidi & Hossain, 2015; Page & Hawes, 2013; Yerima et al., 2012), and although much debate and research continues on the efficacy and safety of extracts, we can still consider certain preparations, as those typically used in medicinal folklore as safe, although again these preparations are artisanal-crafted the potential benefits vary from preparation to preparation. With that in mind, we should not disregard that certain natural compounds can further alter certain pathological states.

Published overtime, we can see a diverse set of studies on Neem aimed to test the antioxidant effect and/or to test the boost of the natural defenses of the body. One such study uses leaves and methanol to extract potential compounds from Neem. In such study, they tested this extract on rats, as a pre-treatment, for 7 days at 100–200 mg/kg, comparing this extract to untreated and vitamin C (a known antioxidant)-treated animal, in a model of induced intestinal ischemic-reperfusion injury (IIRI). IIRI rats reduced expression of extracellular

regulated kinase (ERK1/2), while the extract group reduced several markers of inflammation such as myeloperoxidase in the serum. Similarly, for non-IIRI, nitric oxide levels continued at a steady level (control 0.036 μmole/l, extract 0.034μmole/l and vitamin C 0.042μmole/l), but diminished for IIRI (0.025 μmole/l). Further, extract group increased levels of GSH resulting in the recovery of glucose-6-phosphate dehydrogenase (G6PD), therefore we concluded that the extract helps boost the body's natural defenses (Omóbòwálé et al., 2016).

Other studies using acetic acid to induce a model of colitis in rats, comparing no extract to extract for up to 14 days, confirmed that 14 day treated animals had reduction in colonic mucosal tissue damage and inflammation at both a macroscopic and microscopic level, additionally in this study they measured SOD, CAT and GSH. The colitis model showed an enzyme reduction of 85%, 61%, and 46% respectively, and after treatment, levels of SOD and CAT were almost at the same levels as control, even GSH had recovery levels of 85%. In an interesting development, rats with no extract treatment gained body weight (most likely from inflammatory processes leading to liquid retainment), yet there was no difference in water or food consumption observed when compared to control and extract treated groups. This suggests that the benefits to the natural antioxidant system steams from the consumption of the extract (Gautam et al., 2013; Ghatule et al., 2012).

Other studies., showed that neem enrich yogurts have a higher total phenolic content, up to 20% more when compared to traditional yogurt. This high capacity of enrichment proved valuable, as when laboratory tested Neem enriched yogurt had the capacity for DPPH inhibition of 53.1μgGAE/ml (day 28) vs 35.9 μg GAE/ml as seen on plain yogurt. In addition, they tested for maximum inhibition to key molecules in diabetes and hypertension: α-amylase (47.5%), α-glucoside (15.2%), and angiotensin converting enzyme (48.4%). For all the above, we can conclude that neem enriched yogurt represents a reasonable adjuvant to increase natural scavenging properties within the body (Shori & Baba, 2013).

5. Anti-inflammatory effect

An important property found in Neem extracts is their ability to work as anti-inflammatory agents (Rupani & Chavez, 2018; Soares et al., 2014). Inflammation is a pathophysiological condition involved in a plethora of diseases like cancer and diabetes, as well as in other states such as alcohol consumption and food digestion (Eldeen et al., 2016). Now, a main bioactive compound found in Neem is limonoid. Limonoid is a furanolactone, known for its inhibitory properties in the production of inflammatory mediators, it is also known as a pain anesthetizer, as it stimulates the activation of endogenous opioid pathways (Naik et al., 2014; Schumacher et al., 2011; Soares et al., 2014). Soares *et al.,* showed that limonoid extracted from Neem, can inhibit edema and fibrovascular tissue growth when tested on damage rat paws. They concluded that this was most effective at a dosage of 120 mg/kg, showing particular inhibitory effect over major inflammatory molecules such as tumor necrosis factor alpha(TNF-α) and interleukins (Soares et al., 2014). Over time, several other studies have corroborated and investigated in more detail the mechanism of the anti-inflammatory activity of limonoids (Chen et al., 2018; Kumar, Vidya Priyadarsini, Vinothini, Vidjaya Letchoumy, & Nagini, 2010; Tapanelli et al., 2016; Zhu et al., 2017). To note, much of the conducted research reveals an interesting relation of the anti-inflammatory effects as anti-cancerous agents, more in detail elsewhere in this review. Another interesting compound, with anti-inflammatory effects is epoxy-azadiradione (Fig. 2). This compound shows cytotoxic potential in various pathologies by serving as a modulator of the macrophage migration inhibitory factor; inhibiting its tautomeric activity and the ability of NF-kβ to translocate, preventing the release of proinflammatory cytokines such as IL-1α, IL-1β, IL-6, and TNF-α (Alam et al., 2012; Priyadarsini, Manikandan, Kumar, & Nagini, 2009; Shilpa et al., 2017).

In the body, inflammation leads to the activation of the cyclooxygenase pathway, and the inhibition of cyclooxygenases 1 and 2 (COX1, COX2) by Neem has been a widely studied topic (Someya et al., 2018). We previously mentioned that phytochemical analysis of the Neem oil, confirmed triterpenes as the most important chemical compound found (anti-inflammatory effects) (Naik et al., 2014; Schumacher et al., 2011). We now can relate these compounds to the modulation of inflammation by linking them to eicosanoid metabolism (prostaglandin and thromboxane production), a crucial step is converting arachidonic acid to PGH_2 and further to PGE_2 (Shin & Ava, 2017). This is a conversion mediated by COX2, an enzyme stimulated by IL-1 and by platelet-activating factor, factors expressed in macrophages and monocytes in response to inflammation (Alam et al., 2012; Dayakar et al., 2015). As mentioned before, there is evidence of the anti-inflammatory properties of epoxy-azadiradione and the level of transcription of the NF-kβ, as this factor mediates the production of many inflammatory cytokines, such as IL-1, IL-6 and TNF-α (Alam et al., 2012). Recent studies by Shilpa et al., showed that extracts of Neem could interfere in the IL-1 – COX2 stimulation and producing an antipyretic effect (Shilpa et al., 2017). In addition, also inhibited is NF-kβ's nuclear translocation, therefore reducing the inflammation's overall response. This result is significant as it can serve as a mediator in cancer signaling as it reduces activation of cytokines and TNF-α (Schumacher et al., 2011; Shilpa et al., 2017). By extension, we can conclude that Neem extracts can inhibit inhibitory factors of macrophage migration, responsible for the development of proinflammatory reactions in various diseases (Alam et al., 2012), as exampled by NF-kβ, which directly affect the cells that produce IL-1, IL-2, IL-6, IL-8, IL-12. IL-18, and TNF-α. NF-kβ expression relates to cells of diseases with autoimmune or inflammatory processes, such as monocytes, neutrophils, eosinophils, basophils, blood dendritic cells, B cells and mast cells (Alam et al., 2012; Shin & Ava, 2017).

6. Anti-cancerous effect

Studies over the past several decades, on medicinal plants and phytochemicals (typically present in the diet) continue, in order to determine their anti-cancerous activity (Abdelbaset-Ismail et al., 2016; Arumugam et al., 2014; Cruz-Vega et al., 2009; Hao, Kumar, Yadav, & Chandra, 2014; Nagini, 2014; Patel et al., 2016; Sengupta et al., 2017; Wu et al., 2014). The major aspect normally looked upon is their ability to interfere with multiple pathways that control either growth and/or apoptosis, and even chemo protection (Zhang et al., 2015). One such study was conducted by Pramanik et al. (2016), they tested for the chemo protective of compounds found in Neem, like azadirachtin, nimbolide and limonoid enrich extracts, over models of buccal carcinogenesis in hamsters. They established, that Neem extracts gave positive such as the suppression of the NF-κβ pathway. They further showed the expression profile of proliferating cell nuclear antigen (PCNA), p21, cyclin D1, glutathione S-transferase pi (GST-P), NF-κβ, inhibitor of κβ (Iκβ), p53, Fas, Bcl-2, Bax, Bid, Apaf-1, cytochrome *C*, survivin, caspases-3, -6, -8 and -9 where all tested and results showed their overall reduction (Manikandan, Letchoumy, Gopalakrishnan, & Nagini, 2008). In addition, other researchers have shown prominent anti-cancerous activities from limonoid-derived compounds. Amongst these, both 1-O-deacetylohchinolide B and 15-O-deacetylnimbolindin B are proved to hinder cell growth in human cervical adenocarcinoma (Chen et al., 2018; Kumar et al., 2010; Zhu et al., 2017), by suppression of the NF-κβ, the Wnt/β-catenin and the JAK/STAT pathways (Nagini, 2014). Along these lines, are two more cytotoxic compounds nimbolide and azadirone, both acts to induce ROS mediated apoptosis by inhibiting PI3K/Akt signaling and upregulating reversion-inducing cysteine-rich proteins with Kazal motifs (Hao et al., 2014; Zhu et al., 2017). A fairly new discovered alkaloid-derived limonoid, azadiramide A, is primarily found in Neem leaf ethanolic extracts, shown to stop cell growth and induce apoptosis in both the estrogen independent

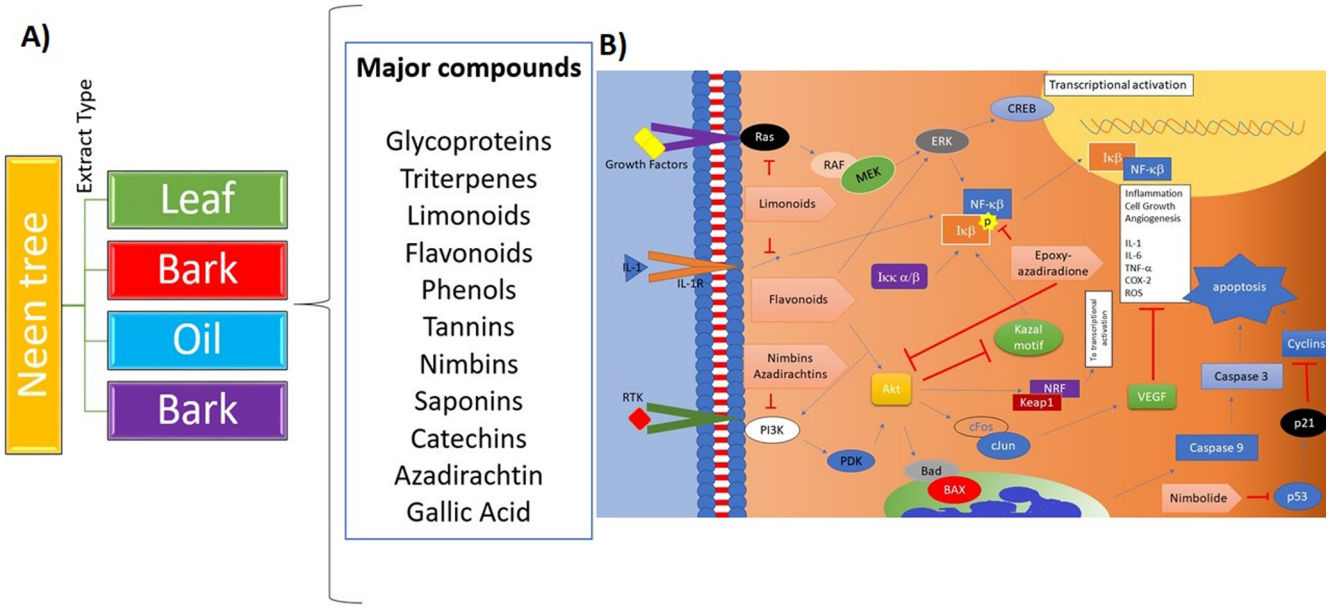

Fig. 2. Neem Tree (Right to left), Types of extracts. Major compounds found in extracts; examples of regulation compounds produce over naturally occurring processes in the human body.

MDAMB-231 and estrogen dependent MCF-7 cell lines of breast cancer in humans (Chen et al., 2018; Elumalai et al., 2012; Zhu et al., 2017). Caspase-3 activity seems to lead the overall apoptotic effect, pro-apoptotic signaling molecules such as Bcl-2 associated X protein (Bax), Bcl-2-associated death promoter (Bad), cytochrome *c*, poly (ADP-ribose) polymerase (PARP) all deemed elevated, while anti-apoptotic protein B-cell lymphoma 2 (Bcl-2), Fas ligand (FasL), Fas associated death domain receptor (FADDR), B-cell lymphoma-extra-large (Bcl-XL) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), were down-regulated when using azadiramide A (Arumugam et al., 2014; Elumalai et al., 2012; Singh, Alex, & Bast, 2014). Further proven were Neem leaf ethanolic extracts in having apoptosis-inducing activity, as they decrease cellular proliferation through the inhibition of IGF signaling molecules (Elumalai et al., 2012; Singh et al., 2014).

Finally, other compounds, such as NLGP, seem to further regulate the activation of NK, NKT and effector T cells. They seem to act upon suppression of the regulatory T cells and continue the modulation of macrophages and antigen-presenting cells through maturation of dendritic cells (Banerjee et al., 2014). They also seem to normalize the immune microenvironment of a tumor, by regulating the balance of cytokines-chemokines (reducing CD31 and VEGFR2) to prevent depletion of effector T cells (Banerjee et al., 2014).

7. Anti-diabetic effect

Diabetes or the lack of control over glucose concentration in the blood is rapidly rising as one of the major chronic degenerative disorders (Hieronymus & Griffin, 2015; Joshi et al., 2010; Shori & Baba, 2013; Upreti, Ali, & Basir, 2013). Conservatively by 2030 there is an expectancy for diabetes to be the 11th leading cause of death worldwide (Mathers & Loncar, 2006). As the disease progresses, it becomes a lifelong burden (physical and economical) over the patient, therefore lower cost treatments become necessary. Among the various methods and pharmacotherapies being developed, the use of Neem extracts has steadily grown in interest (Al Akeel et al., 2017; Joshi et al., 2010; Mathers & Loncar, 2006).

Briefly, there are two types of diabetes. On both types of diabetes, there have been studies of Neem extracts for their effects, with controversial results. We caution the direct use of Neem extracts as they continue being researched for both effects and toxicity. Type I diabetes, known to have an early onset, due to the lack of stemming from the capacity of pancreatic β-cell to produce insulin (Shiuchi et al., 2002). While a combination of a sedentary lifestyle and an excessive caloric intake in genetically susceptive individuals leads to the appearance of diabetes type II, in which insulin resistance is the principal culprit of glucose intake by fat and muscle cells. Under this scenario, a reduction of the glucose-6-phosphate dehydrogenase (G6PD), downregulates the production of NAPDH. The intracellular deduction of NAPDH overtime causes a decline in the antioxidant's effectiveness system and a rampant production of ROS (Abdel-Moneim, Othman, & Aref, 2014; Basir & Shailey, 2012; Ghatule et al., 2012). The overall process disruption introduces a state of oxidative stress, which induces proinflammatory signaling molecules such as TNF-α and IL-6 (Alam et al., 2012; Schumacher et al., 2011). The conclusion of said mechanism is the activation of the insulin resistance pathways, leading to an ultimate diabetic state (Gautam et al., 2015; Singh et al., 2014; Upreti et al., 2013) (Fig. 3).

Several studies carried out in induced-diabetic rat models have revealed rescue of the G6PD when treated with Neem extracts. Specifically, Basir et al., demonstrated retardation in both liver and kidney damage, and recovery in the antioxidative system (Basir & Shailey, 2012; Upreti et al., 2013). They confirmed that both leaf and bark extract had similar glucose homeostasis as compared to standard use of insulin or control. In addition, they showed reestablishment of the SOD, NOD and GSSH function after treatment. Hence, these extracts display an enormous potential as alternative pharmacotherapy (Basir & Shailey, 2012). Further, epoxy-azadiradione enriched extracts purified from the seed of Neem proved an unprecedented effect on glucose levels in diabetic rat models; dropping nearly 37% in a matter of hours. A long-term study devised by Patil et al., showed the effects over a period of 15d, where they could conclude that Neem extracts at 800 mg/kg could modulate the levels of sugar in the blood. Their tested models had glucose levels over 50% could reduce with maintenance of 300 mg/dl during this period. Comparatively, other researchers had similar results when using chloroform-based extracts (Patil, Patil, Mane, & Verma, 2013). These chloroform-based experiments also tested for the recovery effect of G6PD and establish an increase in pancreatic islet function (insulin secretion), resulting in increased levels of glycogen in the muscle and liver (Ghatule et al., 2012; Joshi et al., 2010).

Streptozotocin (STZ) is a potent compound known for its

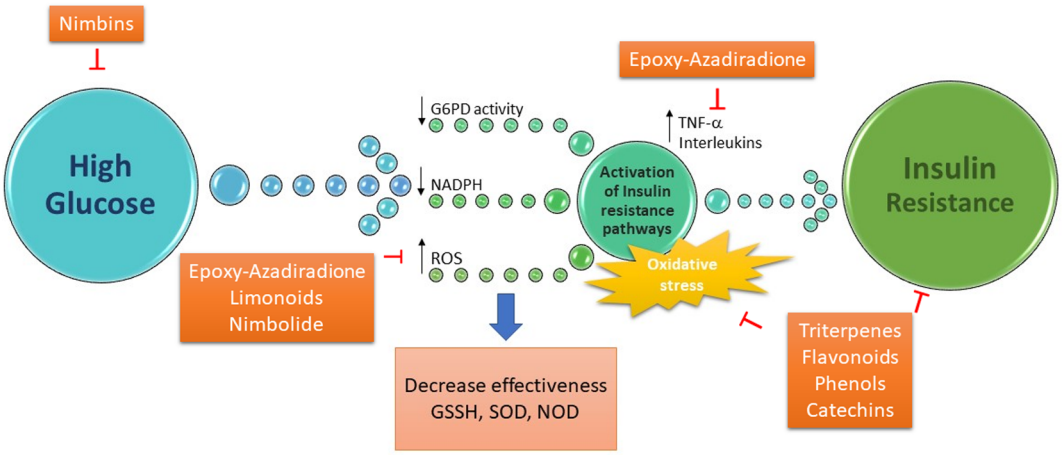

Fig. 3. Insulin resistance progression. Over time as high glucose concentration in present, ROS induced damage is exacerbated and G6PD activity is reduced, thereby reducing the amount of NADPH available. Further oxidative stress is aggravated by overall decrease in the effectiveness of the antioxidant system (GSSH, SOD, NOD) and the induction of pro-inflammatory molecules TNF-a and other cytokines. In addition, the global sum of activities induces in a first instance the activation of the insulin resistance pathways, progressing to a full insulin resistance state.

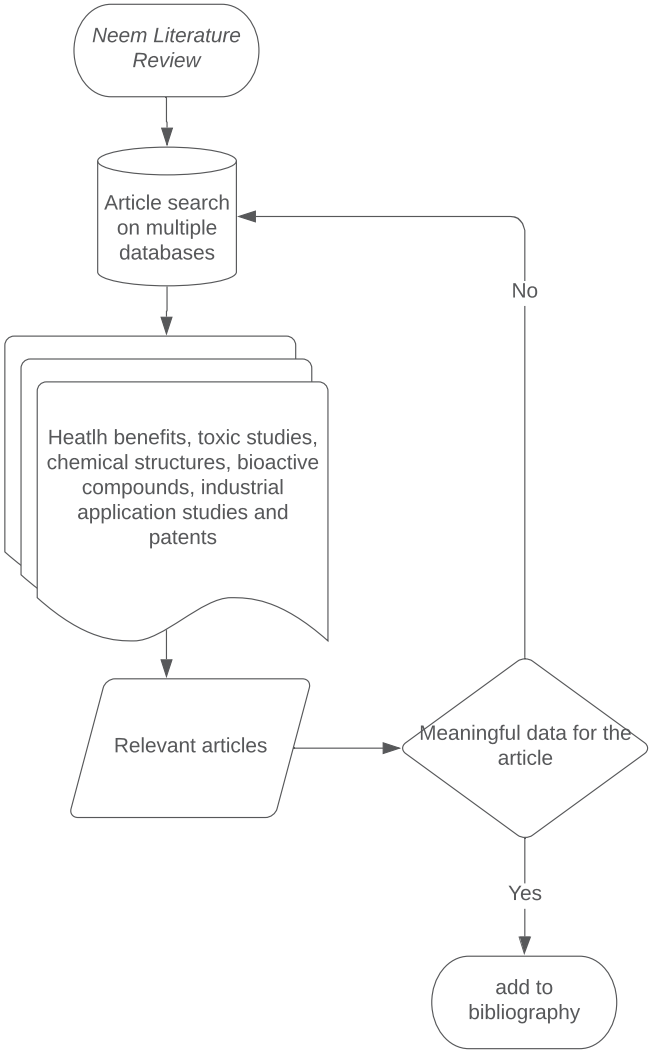

Diagram 1. Methods Flowchart for article and patent searches. Multiple databases were used and articles and patens were screen for meaningfulness based on importance and relevance of literature. Those deemed meaningful were added to our bibliography.

preferential toxicity to β-cells because of the overwhelming induction of methylation and ROS production, and in addition to a gradual decrease in GLUT2 expression (Wang & Glechimann, 1995). For these effects, STZ is a common chemical-inducer of diabetes type 1 in small animals (McCalla, Prashad, Brown, & Gardner, 2015; Upreti et al., 2013). Using Neem extracts on this model have shown some very interesting, yet divisive results. Gardner et al., conducted experiments testing glucose and insulin levels, and islet cell morphology. Their results showed that, after treatment, insulin levels were comparable to those of the control group. They found a striking significance on the cells themselves as regeneration set in. They additionally observed as initial treatments, STZ had obliterated much of the cells, while others had entered a state of reduce and altered morphology and perhaps apoptosis (or necrosis). After treatment there was an increase in total cell migration and granular appearance, but also hypertrophy. Interestingly, not restored are the glucose levels, a phenomenon that seems to go in contrast with what authors mention as previously described antidiabetic effects on β-cells (McCalla et al., 2015). Yet, other researchers have confirmed restorative effects on β-cells when using Neem extract (Hosseini, Shafiee-Nick, & Ghobani, 2015). This controversial state of the art, warrens more detailed and longer-term studies.

As a final comment, we can say that the use of Neem leaf extract compounds have shown positive results in the reduction of glucose and overall pancreatic health as mentioned by McCalla et al, and Patil et al, as well as a retardation of liver and kidney damage, and recovery in antioxidant system mentioned by Basir et al, in murine models, giving an important perspective in the possible use of these compounds. Keeping in mind that diabetes is an important global health disease with a high rate of organ complication such as kidney failure and cardiovascular diseases, the development of new treatments, such as pharmacological active extracts (Neem extracts) to help preserve organ and metabolic integrity holds an important research matter (Basir & Shailey, 2012; McCalla et al., 2015; Patil et al., 2013).

8. Toxicity studies

Recently administered were methanoic leaf extracts to rats, concluding that they had an LD_{50} = 12 g/kg weight. In mice, aqueous extracts presented no toxicity, with LD_{50} = 2 g/kg (Patel et al., 2016). Previous studies conducted acute toxicity tests in rats, via intramuscular injection and using leaves and seed aqueous extracts (Shori & Baba, 2013). Their experiments determined the LD_{50} to be 6.2 and 9.4 mg/kg respectively. Meanwhile, further analysis of the results revealed

Table 1
Summary of results and activities demonstrated both in vivo and in vitro for various types of Neen extracts. These results are classified in accordance to the activity they present and are further separated by various parts of the plants were the extracts were obtained.

Extract (Bark, seeds, leaves, root)	Activity of the extract or compound isolated	In vitro	In vivo	Comments	Ref.
Anti-inflammatory					
Bark	Powdered bark (20 g). Showed ethanolic extract has the highest content of flavonoids and phenols. These compounds have the highest antioxidant activity.	X*		In vitro antioxidant potential	Sultana, Anwar, and Przybylski (2007)
Seeds	Seed oil. A dose of 2 ml/kg body weight extract showed 53.12% inhibition of edema.		X		Naik et al. (2014)
Leaves	Aqueous extract Immunomodulator, growth promoter. Greater weight gain, breast in the 50 ml infusion group. The cost of feeding was significantly higher in the control group than in the Neem group. Greater mortality was observed in the control group. Higher titers of anti-bodies against infectious bursal disease were observed in the group with 50 ml of Neem infusion.		X	Animal study suggesting economical gains with less feed consuption	Durrani et al. (2008)
Leaves	Semisolid extract with methanol. Increase in glutathione levels, better activity of the enzyme G-6-PD.		X	Use of Neem regenerate insulin-producing cells corresponding to increase in the plasma insulin and c-peptide levels	Joshi et al. (2010)
Undefined	Inhibits the proliferative phase of inflammatory response and reduces the growth of fibrovascular tissue. At high doses 120 mg/kg there is effect on the pain receptors, activates endogenous opioid pathways.		X	Demonstrated activity of Neem in reduction of nociceptive and inflammatory pain, by inhibition of inflammation and activation of endogenous opioid pathways	Soares et al. (2014)
ONCOLOGICAL					
Seeds	Cytotoxic. Activity against breast cancer was shown in the MDA-MB231 cell line. 28-deoxo-2,3-dihydronimbolide inhibited the growth activity of the Hela cell line (cervical cancer), A375 melanoma and promyelocytic leukemia HL-60.	X		Neem extrract as a cancer cell modulator of growrth	Chen et al. (2018)
Seeds	Azadiramide inhibits the growth of breast cancer cell line MDA-MB 231.	X		Neem extract as inhibitor of cancer cell growth	Zhu et al. (2017)
Seeds	Cytotoxic Extract extracted through ultrasonication increased effect on the induction of apoptosis in drug-resistant and resistant osteosarcoma cells. The cytotoxicity is attributed to these.	X			Sengupta et al. (2017)
Leaves	Ethanolic extract. Radiotherapy induced binding activity of NF-kB with a relative activation after fractional radiation. Neem leaf extracts significantly inhibited both constitutive and radiotherapy-induced NF-kB. In addition, neem leaf inhibited genes induced by fractionated radiotherapy.	X		Adjuvant for survival after radiotherapy	Alam et al. (2012)
Leaves	Antiangiogenic potential of extract showed control over cell proliferation, attenuation of VEGF and anti-angiogenic effects.	X		Antiangiogenic potential	Omóbòwálé et al. (2016)
Leaves	Suppressed the androgen receptor induced by dihydrotestosterone and prostate-specific antigen levels. The extract inhibited β1 integrin, calreticulin and activated focal adhesion kinase in prostate cancer cells. Oral administration significantly reduced tumor growth of xenograft in mice with formation of hyalinized fibrous tumor tissue and a reduction of prostate-specific antigen and increase in AKR1C2 levels.	X	X	Studies on tumor supression and matrix regulation	Talwar et al. (1996)
Leaves	Ethyl acetate extraction confirms the highest antiproliferative potential.	X			Schumacher et al. (2011)
Leaves	Showed the genetic expression for which they can code for fibroblasts and keratinocytes, before exposure to neem extract.	X			Someya et al. (2018)
Leaves	Raw ethanolic extract. Significantly reduced the incidence of mammary tumors. Neem leaf fraction 10 mg/kg of body weight was effective in the chemoprevention and in the modulation of the enzymatic activities of phase I and II and the oxidant-antioxidant state, inhibiting cell proliferation and inducing apoptosis.		X	Demosntrated higher effect of ethyl acetate over methanolic extracts form Neem. Chemoprotective effects associated to oxidation prevention including DNA damage	Vinothini, Manikandan, Anandan, and Nagini (2009)
Leaves	Extract with ethanol. Inhibits the progression of mammary tumorigenesis induced by chemical carcinogens in rat models. Highly effective in reducing the burden of the breast tumor and in suppressing breast tumor progression, even after cessation of treatment. ↑ p53; ↑ Bax; ↑ Bad; ↑ caspases; ↑ PTEN; ↑ JNK; ↓ Bcl-2; ↓ cyclin; D1; ↓ Cdk2; ↓ Cdk4; ↓ MAPK1.		X	Inhiition of proapoptotic genes using plant-based diets	Arumugam et al. (2014)

(*continued on next page*)

Table 1 (*continued*)

Extract (Bark, seeds, leaves, root)	Activity of the extract or compound isolated	In vitro	In vivo	Comments	Ref.
Leaves	Leaf glycoprotein. Reduction of tumor volume. Temperature is a crucial factor in maintaining the active conformation of the protein, evidence suggests that 56° C preserves the structure. Regarding pH, the restriction was effective when the solution was between 6 and 7.		X	Temperature dependent extraction, demonstrates the capacity on tumor growth restriction	Kundu et al. (2018)
Leaves	Leaf glycoprotein. Restriction of tumor growth, as well as normalization of angiogenesis. The pretreatment facilitates the deep infiltration of CD8 T cells into the tumor parenchyma, which subsequently regulates the VEGF-VEGFR2 signaling in CD31 + vascular endothelial cells to prevent aberrant neovascularization. The following markers were found ↓ CD31; ↓ VEGF; ↓ VEGFR2.		X	NLGP significantly restrict tumor growth and regulate immunomodulation	Banerjee et al. (2014)
Leaves	Immunomodulator aqueous extract. Reduces immunotoxic effect (apoptosis of blood cells) of chemotherapy. It does not stimulate tumor growth or angiogenesis and activates the immune system to restrict tumor growth.		X	Comparison of Neem extract vs GCSF shows Neem extract as a more sustainable tumor growth and angiogenesis inhibitor	Ghosh, Bose, Haque, and Baral (2009)
Leaves	Suppressed the incidence of DMBA-induced carcinomas in hamsters and reduced preneoplastic lesions. Compared with crude extract, fractions of neem leaves showed a greater inhibitory effect on carcinogenesis at an average dose of 10 mg/kg of body weight. The neem leaf fractions function as "double acting agents" by suppressing the activation enzymes of the phase I carcinogen and improving the phase II detoxification enzymes. ↓ PCNA; ↓ Bcl-2; ↑ caspase-3; ↑ PARP; ↓ VEGF.		X	Neem extrract as a tumor cell modulator	Manikandan et al. (2008)
Leaves	Aqueous extract. Decrease tumor incidence in colorectal cancer. ↓Sialic acid.		X	Reduce tumor incidence	Ramzanighara, Ezzatighadi, Rai, and Dhawan (2009)
Leaves	Aqueous extract. There was a reduction in the incidence of tumors by 41.7%. The administration of the extract significantly reduced the levels of bcl-2 and promoted the expression of bax, caspase 3 and caspase 9.		X	Reduce tumor incidence	Arora, Koul, and Bansal (2011)
Ethanolic and aqueous extracts of Neem leaf	Induces apoptosis in colon cancer cells and leukemia, after destabilization of the mitochondrial membrane.		X	Destabilization of mitochondria in tumor cells	Roma et al. (2015)
Undefined	Azadirachtin A, Azadirachtin B, Azadirone (in vitro) produce increased proliferation, differentiation and mineralization in osteoblasts. Azadirachtin A (in vivo) is osteogenic. Stimulating expression of ALP, PunX-2 and CLOL-1 genes at 1 and 5 mg per kg. Accelerates the rate of mineral apposition and bone formation in calvaria cells.	X	X		Baligar et al. (2014)
Undefined	The inhibition of carcinogenesis induced by DMBA by azadirachtin and nimbolide is based on the reduced incidence of preneoplastic and neoplastic lesions; as well as the modulation of xenobiotic metabolizing enzymes, the antioxidant status, 8-hydroxy 2-deoxyguanosine and the markers of invasion and angiogenesis. ↑ GST; QR; ↑ SOD; ↑ CAT; ↑ GSH; ↑ GPX; ↑ GGT; ↑ GR; ↓ MMP-2; ↓ MMP-9; ↓ HIF-1; ↓ VEGF.		X	Anti-cancerous potential of nimbolide	Priyadarsini et al. (2009)
ANTIDIABETIC					
Root	Nimbidin a major active ingredient of Neem seed oil. The root contains both nimbidin and nimbin. Prophylactic agent in diabetes and adjuvant to treatment.	≪	X	Nimbidin activity in diabetes	Patil et al. (2013)
Leaves and bark	Extracts decrease basal plasma glucose, Hb1Ac.	X		Neem extract help modulate levels of glycoxidation	Basir and Shailey (2012)
Leaves	Showed decreased baseline of glucose levels by 36.91%, and decreased serum glucose by 32.18%.		X		Akter et al. (2013)
Leaves	Chloroform extract showed gradual decrease in postprandial glucose over a period of 21 days (antihyperglycemic); controls postprandial hyperglycemia (50% reduction). Increase in G6PD activity. Increased pancreatic islet function to secrete insulin. Increased glycogen level in muscle and liver.		X	Neem extract helps increase overall insulin levels	Joshi et al. (2010)
Leaves	Combined treatment with vanadate and aqueous extract is effective in normalizing altered antioxidant enzymes. Treatment indicates partially corrected hyperglycemia and improved enzyme levels.		X		Upreti et al. (2013)

(*continued on next page*)

Table 1 (*continued*)

Extract (Bark, seeds, leaves, root)	Activity of the extract or compound isolated	In vitro	In vivo	Comments	Ref.
Leaves	Reduces glucose, cholesterol, triglyceride and free radicals in tissue. Demonstrated increase in angiogenesis.		X		Gautam et al. (2013)

* X denotes study type (*invitro* or *invivo*).

hepatocyte degeneration, as the major culprit for animal death, potentially because of the high concentrations of Nimbolide and Nimbic acid in the aqueous extracts. From later studies we know these compounds are toxic to mice, but to a much lesser degree to rats and hamsters (Lisanti, Sajuthi, Agil, & Arifiantini, 2019; Shori, 2012). Interestingly, non-aqueous extract in humans had shown to give rise to skin allergens (Batista et al., 2018; Deng et al., 2013; Patel et al., 2016). This plays into the cautionary note of why so much controversy can and has arisen over the past decades on their use (Akter et al., 2013; Auta & Hassan, 2016; Baligar, Aladakatti, Ahmed, & Hiremath, 2014; Deng et al., 2013).

As for many other traditional extracts, in those derived from Neem, antioxidants seem to be at the forefront as the primary providers of medical properties (Al Akeel et al., 2017; Basir & Shailey, 2012; Shori & Baba, 2013). In the present review, we will analyze this mechanism that would define additional properties, such as anti-inflammation, anti-proliferation (cancer), and antidiabetic. It is of the utmost importance to state that we should all take a cautionary view, with this and with other non-fully characterized natural-occurring compounds in extracts, as being of natural origin does not exclude them from exerting toxic effects. In Fig. 2 we summarize the major compounds found in Neem, and overview the major processes that some these compounds might mediate. In addition, a summary of the results from the major recent studies, using diverse types of extracts, presented in Table 1.

Traditional medical folklore gives rise to the use of many plants and their extracts, as they provide good health to those who use them (Arumugam et al., 2014). Yet this statement hides those cases of lethality, intoxication and concerning side effects that can occur due to the lack of precision in characterizing all compounds found using a specific procedure (Hossain et al., 2013; Kumar et al., 2012). However, toxicity studies, using high precision methods, have helped determine the lethal dose of certain extracts (Deng et al., 2013). In particular, clinical-based studies have revealed that a dosage of Neem oil should be less than 1600 mg/kg/day and should not be administered for a period longer than 90 days (Deng et al., 2013). WebMD, known to contain a summary of medical information, warns directly of a few concerning side effects when ingesting Neem extracts. In-brief, because of lack of research, it considers these extracts as potentially harmful to the liver and kidney. Complementing to how extracts seem to help the immune system activity, we issue a fair warning to its use when known auto-immune diseases are present. Further, all research suggests medical monitoring of medications in particular in blood, as certain medications might interact with compounds present (WebMD, 2018). Researchers have reported hemolytic anemia with jaundice and dizziness after high dosages of herbal intake (Tea) in patients with type-2 diabetes. Although in this case, total discontinuation of other medications was found, the most likely culprit was the excessive intake of the extract (Page & Hawes, 2013). Early animal based studies for congestive heart failure, using IM injections of sodium nimbidate at 250 mg have produced cardiac arrhythmias cautioning their use (Brahmachari, 2004). In humans, studies show severe poising in infants. Extracts from oil ranging from 5 ml upwards to 30 ml demonstrated toxicological effects such as acidosis, drowsiness, seizures, hepatoencephalopathy, and death (Sinniah & Baskaran, 1981; Sinniah, Sinniah, Chia, & Baskaran, 1989). Finally, at an epigenetic level, although almost at a trivial level infertile males treated with Neem have shown a reduction in the methylation pattern of deoxycytidine (Tsarev, 2010).

Unfortunately, the global information found for Neem extracts continues today to be insufficient, as toxicity and side effects are still not well understood. It is consequently sensible not to use these compounds in a liberal, non-restrictive way. Even though centuries of traditions should not be overlooked, thus a righteous balance needed to attain full potentiate the beneficial effects occurring from these natural products and minimizing the possible negative connotations. These extracts should continue under exploration and set for clinical-based trials as an effective, low-cost method to help the overall state of the

patient.

9. Industrialized applications

Through this work, we have mentioned some beneficial roles of Neem studied in models of heart disease, cancer, and diabetes. Such work, along with other beyond this review, has given rise to the development of patentable technology for both clinical and commercial application.

Amongst the various uses of Neem, we begin with its properties as a male contraceptive, as an alternative to vasectomy. A 50ul injection of Neem oil extracted from seeds and applied to the vas deferens in rats shows to block fertility without the loss of libido or androgens. A single dose injection reported to be effective to block fertility over the 9 months of observation (Talwar, Upadhyay, & Dhawan, 1996). In a second instance, Neem oil extracts used as vaginal creams, with spermicide effects, as an effective form of preventing unwanted pregnancies. These creams comprise a mixture of 89:10:1 organic carrier-Neem oil-reetha extract, wherein animals studied showed that 2 ml of cream where enough to prevent pregnancy for up to 3 months with no effect on ovulating cycle. Bonnet Monkey experiments showed similar results 1 in 6 females became pregnant after 50 cycles of mating. Neem creams give rise to immune modulation of TNF-α and γ-interferon primarily affecting placental implantation (Asif, 2013; Talwar, Upadhyay, Kaushic, Singh, & Sharma, 1993). It's important to emphasize is that unlike traditional pill formulations, the use of oil since applied externally does not interfere with hormonal cycle regulation. Khillare et al., proved that a dosage of 3 mg suffices to kill 100% of sperm (1 million in vitro). By direct histological cuts of testis potent spermicide effects are seen, when 100 mg of dried leaf powder suspended in 1 ml of distilled water for a 24 h period. Lower dosages seem to affect the motility of the sperm, hence acting as an ATPase inhibitor (Khillare & Shrivastav, 2003).

Another interesting application is the use of extracts as fungicide (Barnette & Walter, 1997) and insecticide and antibacterial (Barnette & Walter, 1997; Locke et al., 1993, 1995). In both applications, a key compound exploited is the Azadirachtin. Azadirachtin, works to uncouple mitochondrial oxidative phosphorylation, thus inhibiting the respiratory chain. Other components such as nimbidin, nimbin, nimbolide, gedunin, mahmoodin, margolone, and cyclic trisulfide contribute to the antibacterial activity of neem (Al Akeel et al., 2017; Heyman et al., 2017; Khamis Al-Jadidi & Hossain, 2015; Shah et al., 2016). Current commercial applications can be seen mostly for agricultural and residential pest control. We should note pest control alone has an estimated market cap of $16.2 billion and projected to grow upwards of $27 billion by 2025 (GLOBE NEWSWIRE, 2019), making it one of the most rapidly increasing sectors in the market. Surface coating for residential or medical and commercial use can also be found within this sector. Recent applications highlight the need for sterile areas, as such the industrialized market seem to flow toward epoxy-resins and metal or plastic-base covers with a mixture containing natural extracts due to their antimicrobial and antibacterial properties (Forim & Fernandes Da, 2017; Lisec, 2011).

One of the major points referred to during this work is the role of Neem in diabetes. Chauhan et al., developed a novel composition of herbal mix wherein Neem plays a role as a beta cell stimulant enhancing the production of native insulin (Chauhan et al., 2006; Pushpangadan & Prakash, 2006). Furthermore, previous studies had demonstrated that aqueous extract to have properties in glycemic control, showing a reduction of acid phosphate activity and an increase in 5′nucleotidase, an effect attributed to nimbidin amongst other compounds (Puri, 1999). In addition, researchers developed an encapsulated pill containing the extract of neem, which shows an increase in blood antioxidant activity, inhibits LDL oxidation, foam cell formation; all these distinctive of cardiovascular disease. In addition, these preparations also contain high levels of Tannins, which reduce oxidative stress and may prove useful for weight loss (Mazed & Sayeeda, 2011; Naguib, 2004; Tripp, Babish, Bland, Hall, Konda, & Pacioretty, 2012). These applications created around several interesting properties within extracts of neem and have in mind an increase in the overall quality of life.

The inhibition of cancer progression has over time developed new and interesting drugs to target both growth progression and metastasis. Recently, *Azadirachta indica*-derived plant cells were formally introduced as a means of obtaining 17β-hydroxy-17α-methyl-5α-androst-1-en-3-one and 17β-hydroxy-17α-methyl-5α-androstan-3-one. Compounds determined to inhibit NCI-H460 cancer cell line proliferation by means of IL-2 interaction (Saifullah et al., 2012, 2013). Another interesting development has been the development of composition mixtures derived in part by Neem extract to treat cancer. These mixtures appeared to be, inhibitors of topoisomerase I and II, alkylating agents, and microtubule inhibitors (Thompson et al., 2014, 2016). In the early part of the decade, a Japanese group developed a formulation using a combination of Neem, Ganoderma spore, Wanirin, Chaga enzymes and rice, to block the progression of colon cancer to a metastatic state (Tanaka, 2019).

10. Conclusion

While it is comprehensible that not all extracts or compounds derived from Neem-based research will end as a potential drug, or even that some might not even be applicable, due to toxicity. Ítś true that Neem has been used for quite some time to ward off or treat diseases with varying degrees of effectivity. Even today, these treatments based on extracts are used as complementary medicine, and are found to be obtained typically through the artisanal route, and therefore have a lack of reproducibility when producing them. This to highlight the lack of standardization conditions, herein we provided a review of some of the active compounds present in Neem as well as their possible clinical applications.

Studies to date show that Neeḿs most attractive benefits, its anticancerous and anti-diabetic activities, result from the anti-inflammatory properties of the compounds found within. Stopping ROS (anti-oxidant activity) is a measure of prevention and mediation of the potential exacerbation of metabolic diseases. In addition, inflammation is a state reduced by compounds found in neem like limonoid. Limonoid, an example of compounds which not only reduce inflammation but also work as a pain-relief agent, due to the activation of the opioid pathway. Regulating the activity of T, NK and NKT cells by NLGP and pro-apoptotic signaling molecules like caspase-3 with inhibition of different inflammatory cytokines, have a significant impact in preventing disease such cancer. In a similar fashion the regulation of proinflammatory signaling it is also shown to have profound effects on diabetes. Un regulated levels of ROS overtime disrupts the homeostasis of g6pd leading to a reduction of NADPH. In time, this becomes a cyclic process as this reduction leads again to activations of proinflammatory molecules and activation of insulin resistance.

An important archive of information is present in the literature about these issues, but important gaps that appeals to the scientific rigor and concise research methodology are lacking, even with the presence of patents. Important gaps are present with regards to good manufacturing practice (GMP) obtention of the different compounds, precise dose determinations, and more importantly, to test their true effectiveness by means of randomized double-blind studies.

Finally, we should make a note that extracts derived from neem also have other properties, which affect the industrial markets, such as their potential as fungicides, bactericides, and surface coats (medical, residential, and commercial), with an estimated a market cap close the $20 billion. Therefore, the wide variety of neem and its extracts extend beyond traditional medical folklore. By making use of scientific and technological advance we can now use these extracts as current medical adjuvants, understanding their potential. Expanding on the

development of knowledge, we now know how-to even give them further industrial applications.

11. Ethics statement

Research did not include any human subjects and animal experiments.

12. Authors' contributions

Research and Writing: E.E.A.-F., J.F.I., Z.G-B; Analysis and Edition: J.E.M.-C., Z.G-B, B.A.E.-A; Supervision: J.F.I., J.E.M.-C., B.A.E.-A., M.G.M.-T.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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