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Reviewv

Current evidence about Neem extracts, the molecules and physiological mechanisms their nutritional components interact with.

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Abstract: Plant-based alternative medicine is normally grounded on empiric cultural perception, as the main objective, these practices intent to either maintain good health or to provide a route to turn-the-tide on a specific disease or ailment. Amongst the thousands of plants that have been used and studied, Neem *(Azadirachta indica)* seems to have a very interesting tale to tell, since its properties to ward-off certain diseases have overtime, and in a rigorous way, been proven. The preceding concise review is a collection of some of the most relevant studies today, not only focusing on the health benefits obtained by its use, but digging into the molecular mechanisms of how the properties come about. In particular, we take a look over antioxidant properties and how these mediate and mitigate important molecules such as IL-6 and TNF- , leading the way in reducing systematic damage by oxidative stress. Further, we relate this oxidative reduction to other systemic diseases such as cancer and diabetes, as these are currently becoming the most rampant killers. As of yet not all is known about the different ways of extracting or the total composition of an extract, as these may be from different parts of the plant. Therefore, we also allude to an important cautionary view where toxicological effects and conflicting outcomes arise. Overall, presented results show a great potential for the different extracts of Neem as their antioxidant activity can be taken advantage off, and potentially used in modern medicine.

Keywords: Neem extracts; Nutritional components; Systemic diseases

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 [1]. Historically, ancient Sanskrit had a particular expression for this state, known as "*Nimba"* [2]. Over time the term Neem, derived from *Nimb*a, was used to reference the *Azadirachta indica* (Neem) tree, as its extracts where used to bring "good health" [1,3,4]. The Neem tree, primarily cultivated in southern regions of Asia, has been used for many ages in medical folklore to treat diseases [1,3,4]. In a global scale, the use of various forms of alternative medicine as primary care is estimated to be around 80% in developing countries [5]. Meanwhile in developed (or industrialized) countries, the use of alternative medicine has gained popularity as a complementary way of care. An effect mostly attributed to migration; as people move towards more developed countries they bring not only their skills, but their traditions and way of life [6]. In today's modern world, places like India, Pakistan, and other eastern developing countries [7,8],

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the use of Neem extracts to ward-off certain diseases continues to persist. Tradition has shown, through different generations the beneficial properties of this plant [9–11]. Therefore, it is unsurprising that several parts of the Neem tree have been taken to produce extracts. Out of all the noticeable parts, the oil appears to be the most widely used portion [4,6]. Nonetheless, the use of oil brings forth a few major drawbacks, in particular since the lack of information regarding toxicity levels and full characterization have not yet been fully derived. This plays into the cautionary tale of why so much controversy can and has arisen over the past decades on their use [6,12–14].

As is the case for many other traditional extracts, in those derived from Neem, antioxidants seem to be at the forefront as the main providers of medical properties [10,15,16]. In the present review, we will dive into important molecular mechanisms that also define additional properties, such as anti-inflammatory, anti-proliferative (cancer), and antidiabetic. It is of the upmost importance to state that a cautionary view should be taken, with this as well as with other non-fully characterized natural-occurring compounds in extracts, as being of natural origin does not exclude them from exerting toxic effects. In Figure 1 we will summarize the major compounds found in Neem, as well as overview major processes that these compounds can potentially mediate. In addition, a summary of the results from the major recent studies using diverse types of extracts are presented in Table 1.

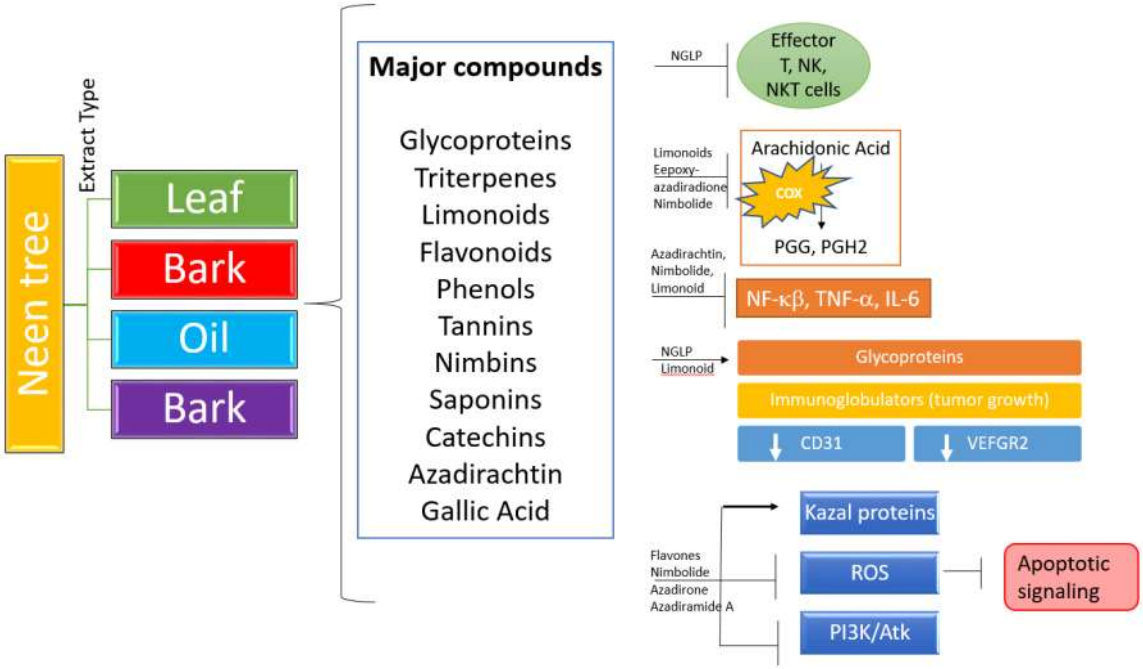

Figure 1. Neem Tree (Right to left), Types of extracts. major compounds found in extracts; examples of regulation compounds produce over naturally occurring processes in the human body.

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Table 1. Summary of results and activities demonstrated both in vivo and in vitro for various types of Neen extracts. These results are best classified in accordance to the activity they present and are 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	Ref.
	ANTI-INFLAMMATORY			
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	[35]
Seeds	Seed oil. A dose of 2 ml / kg body weight extract showed 53.12% inhibition of edema.		X	[18]
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	[20]
Leaves	Semisolid extract with methanol. Increase in glutathione levels, better activity of the enzyme G-6-PD.		X	[3]
Bark	Powdered bark (20 g). Showed ethanolic extract has the highest content of flavonoids and phenols. These compounds have the highest antioxidant activity.	X		[73]
	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		[40]
Seeds	Azadiramide inhibits the growth of breast cancer cell line MDA-MB 231.	X		[39]
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	[74]
Leaves	Extract with ethanol. Inhibits the progression of mammary tumorigenesis induced by chemical carcinogens in rat models. Highly effective in reducing		X	[1]

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	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.		
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 to 7.	X	[22]
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	[21]
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	[75]
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	[53]
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	[41]
Leaves	Aqueous extract. Decrease tumor incidence in colorectal cancer. ↓ Sialic acid.	X	[76]
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	[77]

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Ethanolic and aqueous extracts of Neem leaf	Induces apoptosis in colon cancer cells and leukemia, after destabilization of the mitochondrial membrane.		X	[78]
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		[47]
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		[79]
Leaves	Antiangiogenic potential of extract showed control over cell proliferation, attenuation of VEGF and anti-angiogenic effects.	X		[80]
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	[51]
Leaves	Ethyl acetate extraction confirms the highest antiproliferative potential.	X		[81]
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	[82]
Leaves	Showed the genetic expression for which they can code for fibroblasts and keratinocytes, before exposure to neem extract.	X		[44]
	ANTIDIABETIC			
Leaves	Showed decreased baseline of glucose levels by 36.91%, and decreased serum glucose by 32.18%.		X	[13]
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	[83]
Leaves	Chloroform extract showed gradual decrease in postprandial glucose over a period of 21 days (antihyperglycemic); controls postprandial hyperglycemia		X	[7]

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	(50% reduction). Increase in G6PD activity. Increased pancreatic islet function to secrete insulin. Increased glycogen level in muscle and liver.			
Leaves and bark	Extracts decrease basal plasma glucose, Hb1Ac.	X		[15]
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	[58]
Leaves	Reduces glucose, cholesterol, triglyceride and free radicals in tissue. Demonstrated increase in angiogenesis.		X	[25]

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2. Bioactive compounds present in *Azadirachta indica*

Overtime, research has shown that triterpenes lead the way as the key compounds [17,18], with other important-derived metabolites like: flavonoids, limonoids, saponins, tannins (Azadirachtin and nimbins), catechins and gallic acid [17,18], as seen on Figure 2. In addition, the leaf of the Neem tree appears to have developed a particular set of glycoproteins: such as neem leaf glycoprotein (NLGP) that when tested on mammalian subjects, show important immune-modulators, providing the potential to restrict tumor growth by modulating local and systemic immunity [19–22]. Further, in-depth phytochemical analysis of the oil, has confirmed triterpenes, flavonoids and saponins as the primary compounds found, while other components such as: catechins and nimbins, seem to be present in lower amounts [17,18].

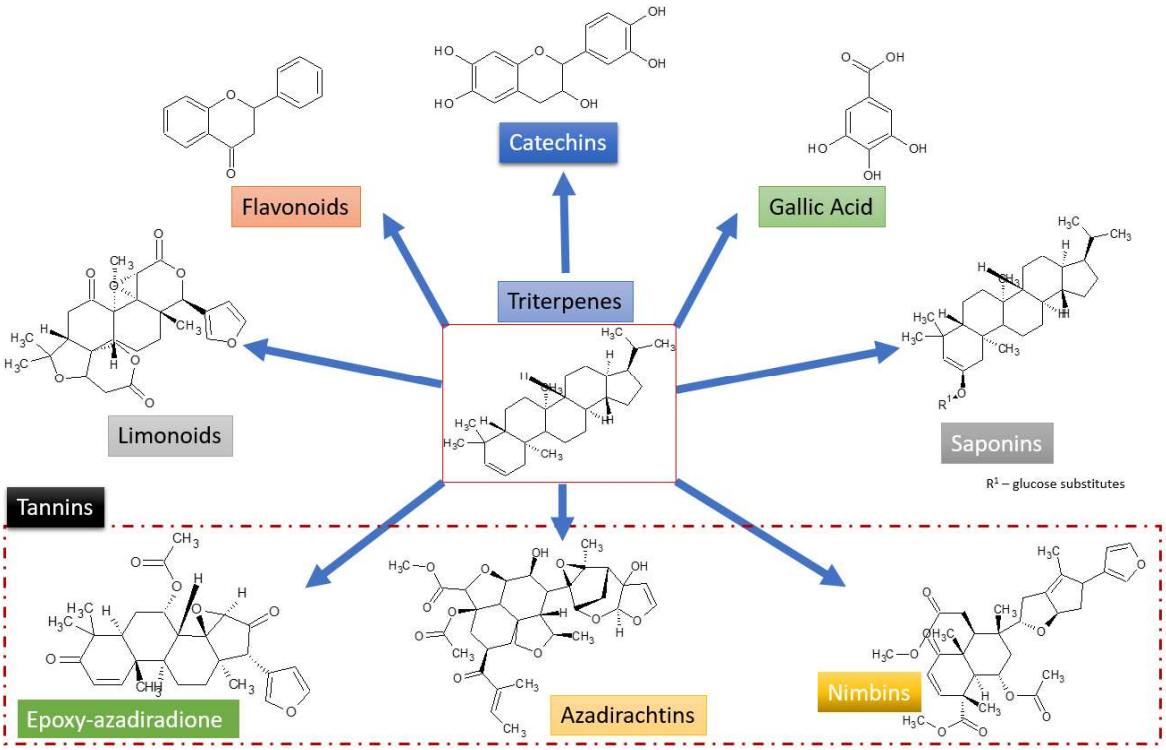

Figure 2. Mayor metabolites found in Neem. Triterpenes are known to be the primary structure on which the major metabolites as founded on. Deriving from these compounds, the Neem tree produces: Flavonoids, Limonoids, Tannins in particular Epoxy-azadiradione, Azadirachtin, and Nimbins, also found in Neem are Saponins, Gallic Acid, and Catechins.

3. Antioxidant effect

Free radicals or reactive oxygen species (ROS) are known to be 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, thereby unleashing in the cell a state of oxidative stress [23,24]. Clearly, the need for providing adequate compounds to stabilize or neutralize these radicals is paramount as a step in preventing or blocking an exacerbation of diseases; as these molecules will add in a positive way to the body's natural antioxidant defenses: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione (GSH), nitric oxide dioxygenase (NOD) [15,25]. A simple way to provide such compounds is to supplement them in the diet, and as such, natural extracts like those derived from Neem seem to be a cost-effective way to introduce them [11,23,26–28].

Overtime a diverse set of studies on Neem have been aimed to test antioxidant effects and/or boost the natural defenses of the body. One such study uses leaves and methanol to extract potential compounds from Neem. This extract was then tested on rats, as a pre-treatment for a period of 7 days at 100 – 200 mg/kg, comparing either extract or vitamin C before intestinal ischemic-reperfusion

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injury (IIRI). Untreated or control rats reduced expression of extracellular regulated kinase (ERK1/2), whereas in both treated cases levels were maintained. Additionally, other markers of inflammation, such as myeloperoxidase in the serum, were reduced by more than half in the extract group when compared to the IIRI only. In a similar fashion, nitric oxide levels were maintained (control 0.036 mole/l, extract 0.034 mole/l and vit c 0.042 mole/l), but diminished for the IIRI (0.025 mole/l). Furthermore, levels of GSH were increased resulting in high recovery of the glucose-6-phosphate dehydrogenase (G6PD), therefore boosting the body's natural defenses [3]. Furthermore, studies by Ghatule et al., used acetic acid to induce colitis in rats. They demonstrated a reduction in colonic mucosal tissue damage and inflammation at both a macroscopic and microscopic level, upon a 14-day use of Neem extract (50% ethanolic). SOD, CAT and GSH were also measured. Colitis model showed a decrease of 85%, 61%, and 46% respectively. Whereas after treatment, levels of SOD and CAT where verily undistinguishable from the control, while GSH had an astonishing 85% recovery. In an interesting development, rats with no extract treatment began to take on 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, suggesting benefits in overall health steaming from the consumption of the extract [29,30].

Yogurt is known to be a healthy alternative food source, in part due to its easy digestibility, as well as its high bioavailability of many nutrients [31]. Preparations can and are normally enrich from a wide array of nutritional sources. Neem based extract yogurt, has been recently studied and comparative analysis has proven that not only Neem-enriched yogurt vs plain yogurt maintains a better pH, but it can increased 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging (considered the gold standard when testing for antioxidant activity) [24,32–34]. Initial studies from Shori et al., demonstrated that Neem-enrich yogurt have a higher total phenolic content (20%) when compared to traditional yogurt. This high capacity of enrichment proved valuable as the inhibition of DPPH was 53.1 gGAE/ml (day 28) vs 35.9 gGAE/ml as seen on plain yogurt. In addition, the Neem variety was tested for maximum inhibition to key molecules in diabetes and hypertension: -amylase (47.5%), -glucoside (15.2%), and angiotensin converting enzyme (48.4%). Hence demonstrating to be a good adjuvant to regular medical treatment [16]. As we continue through the importance of extracts, we will be revealed specific compounds and their effects on diseases such as cancer and diabetes underlining the important molecules they can regulate.

4. Anti-inflammatory effect

Standing out as the paramount property found in Neem extracts, is their ability to work as anti-inflammatory agents [5,35]. 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 [36]. 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 known as a pain anesthetizer, as it stimulates the activation of endogenous opioid pathways [17,18,35]. Soares *et al.*, showed that, limonoid extracted from Neem, can inhibit edema and fibrovascular tissue growth when tested on damage rat paws. Further, they concluded that the extract administered at 120mg/kg had an inhibitory effect over major inflammatory molecules such as tumor necrosis factor alpha (TNF- and interleukins [35]. Over time, several studies have corroborated and investigated in more detail the mechanism of the anti-inflammatory activity of limonoids [37–40]. To note, much of the conducted research reveals an interesting relation of the anti-inflammatory effects and the downstream result as anti-cancerous agents, as described elsewhere in this review. In many instances this effect is by inhibition on effects of reactive oxygen species (ROS)[40]. Another interesting compound, epoxy-azadiradione (Figure 2) seems to show a great cytotoxic potential in various pathologies by serving as a modulator of the macrophage migration inhibitory factor, by inhibiting its tautomeric activity and the ability of NF- to translocate, thereby preventing the release of proinflammatory cytokines such as IL-1α, IL-1β, IL-6, and TNF-α [41–43].

In the body, inflation lead 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 [44]. We previously

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mentioned, in an in-depth phytochemical analysis of the Neem oil, confirmed the presence of triterpenes as the most important chemical compound found (anti-inflammatory effects) [17,18]. We can now relate these compounds to the modulation of inflammation by relating to the eicosanoid metabolism (prostaglandin and thromboxane production). A crucial step is converting arachidonic acid to PGH_2 and further to PGE_2 [45]. This conversion is mediated by COX2, an enzyme that is stimulated by IL-1 and by platelet activating factor. A factor expressed in macrophages and monocytes in response to inflammation [19,42]. As mentioned before, there is evidence of the anti-inflammatory properties of epoxy-azadiradione and the level of transcription of the NF- , as this factor mediates the production of many inflammatory cytokines, such as IL-1, IL-6 and TNF-α [42]. Recent studies by Shilpa *et al.*, demonstrated that extracts of Neem could interfere in the IL-1 – COX2 stimulation and producing an antipyretic effect [43]. In addition, NF- 's nuclear translocation seems to also be inhibited, thereby reducing 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- [17,43]. By extension, Neem extracts can inhibit the inhibitory factor of macrophage migration, responsible for the development of proinflammatory reactions in various diseases such as sepsis, diabetes mellitus, glomerulonephritis, psoriasis, rheumatoid arthritis, lupus, atherosclerosis, inflammatory bowel disease, gastric ulcer, among others [42]. This factor is found to affect the cells that produce IL-1, IL-2, IL-6, IL-8, IL-12. IL-18, and TNF-α and is expressed in different types of cells that are related to diseases with autoimmune or inflammatory processes, such as monocytes, neutrophils, eosinophils, basophils, blood dendritic cells, B cells and mast cells [42,45].

5. Anti-cancerous effect

Over the past several decades, an endless count of medicinal plants and phytochemicals (typically present in the diet) have been studied to determine their anti-cancerous activity [1,4,46–51]. The major aspect normally looked upon is their ability to interfere with multiple pathways that control either growth and/or apoptosis [52].

For the particular case of the Neem, Pramanik *et al.*, evaluated the chemo protective effect of azadirachtin, nimbolide and limonoid enrich extracts, over models of buccal carcinogenesis in hamsters. They demonstrated positive effects after using these extracts, due to the overall suppression of the NF-κ pathway. Expression profile of proliferating cell nuclear antigen (PCNA), p21, cyclin D1, glutathione S-transferase pi (GST-P), NF-κ , inhibitor of κ (Iκ), p53, Fas, Bcl-2, Bax, Bid, Apaf-1, cytochrome C, survivin, caspases-3, -6, -8 and -9 where all evaluated and shown to be reduced [53]. In addition, other researchers have shown prominent anti-cancerous activities from limonoid-derived compounds. Amongst these, it is noteworthy to mention that both 1-O-deacetylohchinolide B and 15-O-deacetylnimbolindin B have been demonstrated to hinder cell growth in human cervical adenocarcinoma [38–40], by achieving suppression of the NF-κ , the Wnt / β-catenin and the JAK / STAT pathways [46]. Along these lines, two more cytotoxic compounds have been widely studied: nimbolide and azadirone [39,49]. Both of these, act to induce ROS mediated apoptosis by inhibiting PI3K/Akt signaling, and upregulating reversion-inducing cysteine-rich proteins with Kazal motifs. These compounds are novel tumor suppressors, determined to present broad inhibitory effects on cancer cell growth [39,54]. Furthermore, a fairly new discovered alkaloid-derived limonoid, azadiramide A, primarily found in Neem leaf ethanolic extracts, has been shown to stop cell growth and induce apoptosis in both the estrogen independent MDAMB-231 and estrogen dependent MCF-7 cell lines of breast cancer in humans [39,40,55]. 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) were 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 [1,55,56]. Further, Neem leaf ethanolic extracts have also been proven to have apoptosis-inducing activity. A conclusion observed, as they seem to decrease cellular proliferation through the inhibition of IGF signaling molecules [55,56].

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Finally, other compounds such NLGP, seem to further regulate the activation of NK, NKT and effector T cells. Additionally, 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 [21]. Furthermore, 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.[21]

6. Anti-diabetic effect

Diabetes or the lack of control over glucose concentration in the blood is rapidly arising as one the major chronic degenerative disorders [7,16,57,58]. Conservatively, by 2030 diabetes is expected to be the 11th leading cause of death worldwide [59]. 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 currently being developed, the use of Neem extracts has steadily grown in interest [7,10,59].

Briefly, there exist two main types of diabetes. On both types of diabetes, Neem extracts have been studied for their effects, with controversial results. Type I diabetes in known to have an early onset, due to the lack stemming from the capacity of pancreatic -cell to produce insulin [60]. While a combination of a sedentary life-style and an excessive caloric intake in genetically susceptive individual, 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), spearheads a decrease in the production of NAPDH. The intracellular deduction of NAPDH overtime causes a decline in the effectiveness of the antioxidant system and a rampant production of ROS [15,30,61]. The overall process disruption introduces a state of oxidative stress, which in turn induces proinflammatory signaling molecules such as TNF and IL-6 [17,42]. The conclusion of said mechanism, is the activation of the insulin resistance pathways, leading to a final diabetic state [25,56,58] (Figure 3).

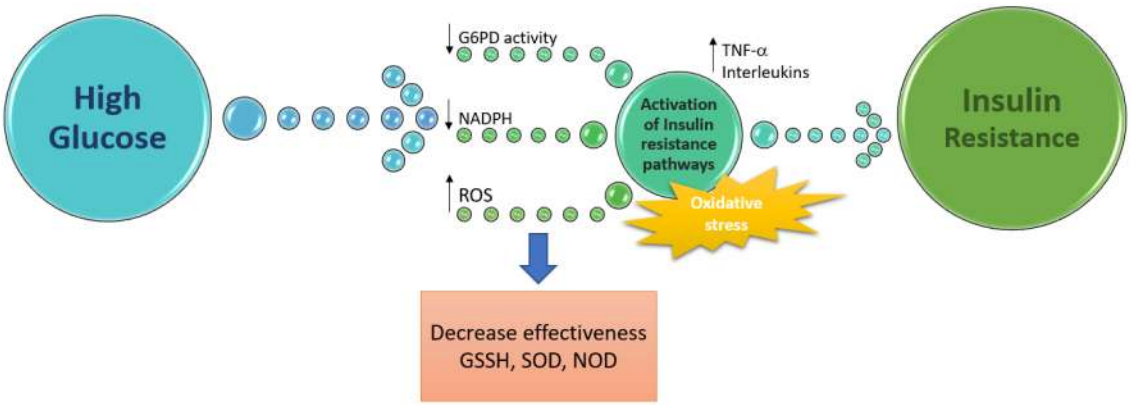

Figure 3. Insulin resistance progression. Over time as high glucose concentration in present, ROS induced damage is exacerbated and G6PD activity is reduced, thereby reducing the amount of NADPH available. Further oxidative stress is aggravated by overall decrease in the effectiveness of the antioxidant 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.

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, as well as recovery in the antioxidative system [15,58]. They were able to

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demonstrate 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 [15]. Further, epoxy-azadiradione enrich extracts purified from the seed of Neem demonstrated an unprecedented effect on glucose levels in diabetic rat models; dropping nearly 37% in a matter of hours [8]. A long-term study devised by *Patil et al.*, on demonstrated the effects over a period of 15d, where they were able to conclude that Neem extracts at 800mg/kg could modulate the levels of sugar in the blood. Their tested models, had glucose levels over 300 mg/dl and could be reduce and maintained by over 50% during this period [9]. Comparatively, other researchers had similar results when using chloroform-based extracts. Additionally, these chloroform-based experiments also tested for the recovery effect of G6PD and established increase in pancreatic islet function (insulin secretion), resulting in increased levels of glycogen in the muscle and liver [7,30].

Streptozotocin (STZ) is a potent compound known for its preferential toxicity to -cells due to the overwhelming induction of methylation and ROS production, in addition to a gradual decrease in GLUT2 expression [62]. Due to these effects, STZ is a common chemical-inducer of diabetes type 1 in small animals [58,63]. Thus far, using Neem extracts on this model have shown some very interesting, yet divisive results. In particular, Gardner *el al.*, conducted experiments evaluating glucose and insulin levels, as well as islet cell morphology. Their results showed that, after treatment, insulin levels were comparable to those of the control group. Furthermore, a striking significance was found on the cells themselves as regeneration set in. This was observed as initial treatments of STZ had obliterated much of the cells, while others had entered a state of reduce and altered morphology and perhaps apoptosis (or necrosis). Nonetheless after treatment there was an overall increase in total cell migration and granular appearance, but also hypertrophy. Interestingly enough, glucose levels did not seem to be restored. This phenomenon seems to go in contrast with what authors mention as previously described antidiabetic effects on -cells [63]. Yet, other researchers have confirmed restorative effects on -cells when using Neem extract [64]. This controversial state of the art, warrens more detailed and longer-term studies to best overall confirm such properties.

7. A cautionary tale

Traditional medical folklore gives rise to the use of many plants and their extracts, as they provide good health to those who use them [1]. 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 [65,66]. However, toxicity studies using high precision methods, have help determine the lethal dose of certain extracts [6]. 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 [6]. WebMD, known to contain a summary of medical information, warns directly of a few concerning side effects when ingesting Neem extracts. In-brief, due to lack of more research it considers these extracts as potentially harmful to the liver and kidney. Complementing to how extracts seems to help the immune system activity, a fair warning is issued to its use when known auto-immune diseases are present. Further, it is suggested to monitor medications in particular in blood, as certain medications might interact with compounds present [67]. Hemolytic anemia with jaundice and dizziness has been reported after high dosages of herbal intake (Tea) in patient with type-2 diabetes. Although in this particular case a total discontinuation of other medications where also found, the most likely culprit was the excessive intake of the extract[28]. Early animal based studies using IM injections of sodium nimbidate at 250mg have been used for congestive heart failure, yet intravenous Neem extracts have proven to produce cardiac arrythmias cautioning their use [68,69]. In humans severe poising has been reported in infants. Extracts from oil ranging from 5ml upwards to 30ml demonstrated toxicological effects such as: acidosis, drowsiness, seizures, hepatoencephalopathy, and death [70,71]. Finally, at an epigenetic level, although almost at a trivial level infertile males treated with Neem have shown a reduction in methylation pattern of deoxycytidine[72].

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8. Conclusion

Systemic diseases such as cardiovascular-related diseases, cancer and diabetes seem to be rapidly rising as the most likely cause of death worldwide. At the frontline of these diseases, recognizable effects of ROS are present. Much of the effects by ROS can be observed primarily on damaging DNA, proteins and also other biological compounds. Therefore, not only inducing but exacerbating these upward mentioned diseases; the mechanisms to overcome them become overwhelmed and battered by the addition of inflammation. As we have seen over the course of this concise review, inflammation, in particular activity lead by TNF- and NF- signaling, once hyperactivated leads into a prolonged and cycling state. Consumption of phytochemical antioxidants found in plants, seems to be a simple and effective way to help boost the body's way to overcome inflammatory effects. This is due to a dual effect of easy absorption of these molecules and a boost of their scavenging properties. *Azadirachta indica* colloquially known as Neem, has been since ancient times and in various cultures, a source of these antioxidant molecules. Overtime researchers have begun to characterize the compounds found in the various Neem extracts, as ways to best understand their clinical potential; and as we have seen, in a wide variety of cases, compelling experimental evidence suggests that both the myriad (full extracts) and the isolated compounds have a wide range of effects, *e.g.*, limonoids as pain anesthetizers, as well as modulators in adenocarcinomas, glycoproteins as immunomodulators, nimbolides as anticarcinogenic and proapoptotic.

Unfortunately, the global information found for Neem extracts continues today to be insufficient, as toxicity and side effects are still not extensively worked out. It is consequently sensible that these compounds not be used in a liberal non-restrictive way. Century of traditions cannot be overlooked, thus a righteous balance needed to be attained in order to fully potentiate the beneficial effects occurring from these natural products and minimizing the possible negative connotations. These extracts should continue to be explored and set for clinical-based trials, in particular, as an effective, low-cost method to help the overall state of the patient.

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