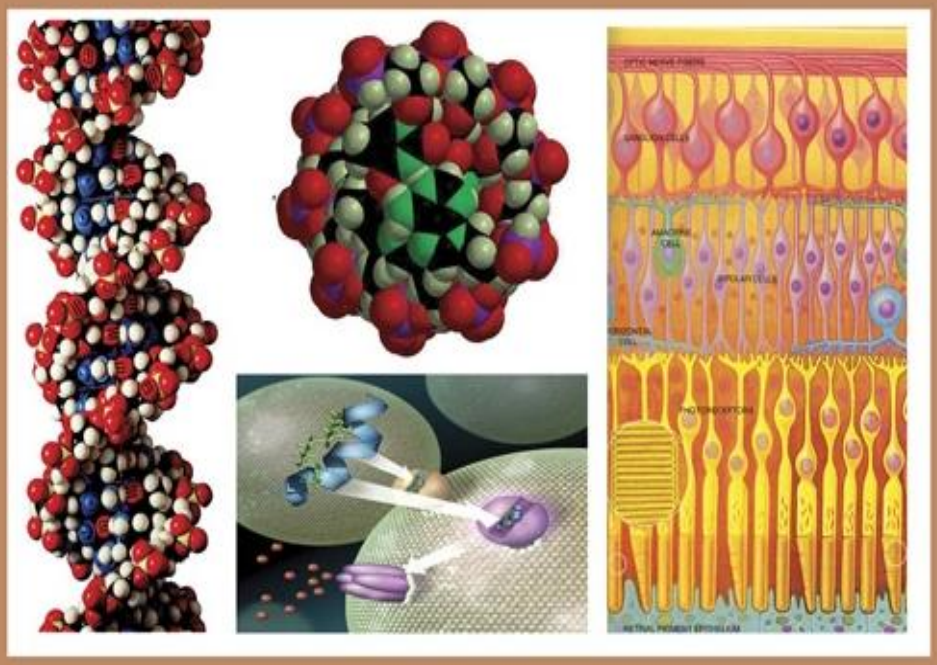
EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
C
PHYSIOLOGY & MOLECULARBIOLOGY



ISSN
2090-0767

WWW.EAJBS.EG.NET

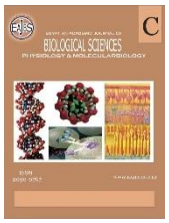
Vol. 15 No. 1 (2023)

.

Citation: *Egypt.Acad.J.Biolog.Sci. (C. Physiology and Molecular biology) Vol. 15(1) pp429-437 (2023)*
DOI: 10.21608/EAJBSC.2023.300377

Egypt. Acad. J. Biolog. Sci., 15(1):429-437 (2023)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



A Review on: Ashwagandha Root Extract Phenolic Compounds Counteract Alloxan's Effects on Oxidative Stress, Inflammation, and Peripheral Nerve Injury

Heba A. Hashem; Zohour I. Nabil and Heba N. Gad EL-Hak
Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.
***E-mail:** hebahashemm@gmail.com- zohournabil@yahoo.com-heba_ahmed@science.suez.edu.eg

REVIEW INFO
Review History
Received:17/4/2023
Accepted:19/5/2023
Available:25/5/2023

Keywords:
Ashwagandha; Diabetes; Oxidative stress; Inflammation; Diabetic neuropathy.

ABSTRACT

Diabetes mellitus is the most common chronic metabolic disorder that progresses slowly and silently. As hyperglycemia becomes chronic with time, it leads to serious consequences in several tissues, especially those that are insulin-insensitive (retina, neurons, kidneys). Although synthetic oral hypoglycemic drugs alongside insulin are the main route for controlling DM, they fail to reverse the course of its complications completely and further worsen it by the fact that they also demonstrate prominent side effects. Ashwagandha (*Withania somnifera*) is one of the most important herbs of Ayurveda "the traditional system of medicine in India. Ashwagandha root and leaf extract demonstrated a hypoglycemic activity comparable to the standard drug glibenclamide in alloxan-induced diabetic rats. Due to its neuroprotective properties, preclinical and clinical studies have supported the use of ashwagandha for the treatment of a wide range of neurological conditions. Ashwagandha has strong antioxidant properties, which aid in the prevention of cellular damage caused by free radicals.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic syndrome characterized by chronic hyperglycemia due to impaired carbohydrate, fat, and protein metabolisms caused by either a lack of insulin secretion or a decrease in tissue sensitivity to insulin (Ozougwu *et al.*, 2013). In the long run, hyperglycemia can lead to a variety of complications such as retinopathy, nephropathy, neuropathy, and cardiovascular disease (Poznyak *et al.*, 2020). According to Zochodne (2007), DM is a major cause of nerve damage, especially in the longer peripheral nerves that innervate the lower limbs. One of the mechanisms involved in diabetic-induced neuronal damage is most likely oxidative stress (OS) (Vincent *et al.*, 2004). The brain is thought to be more vulnerable to OS as it consumes a high amount of oxygen, has a high level of polyunsaturated fatty acids, and has a low level of antioxidant enzymes (Singh *et al.*, 2019). Under diabetic conditions, brain damage clearly got worse (Wang *et al.*, 2001). Martínez-Tellez *et al.* (2005) indicated that DM is linked to structural, morphological, and functional changes in the brain. Furthermore, DM is widely regarded as an inflammatory disease, in which different cytokine concentrations have been found to rise in the serum of diabetic patients (Deng and Chow, 2010). In fact, OS and neuro-inflammation have been identified as key pathophysiological triggers in a variety of diabetes-related microvascular complications, including DN (Abdel-Hay *et al.*, 2018).

In recent years, the medical system has continued to face difficulties in managing DM without any side effects. Along with insulin, a variety of oral synthetic hypoglycemic medications are available for the treatment of DM with their side effects (Banerjee *et al.*, 2020). As a result, there is an increase in demand for natural products that have anti-diabetic activity with fewer side effects. *Withania somnifera* (Family: Solanaceae), also known as ashwagandha, is widely used in India's Ayurvedic system of medicine (Visweswari *et al.*, 2013). Several studies on this plant have revealed that it has anti-diabetic, anti-oxidative, anti-inflammatory, antitumor, anti-stress, immunomodulatory, and hematopoietic properties (Alam *et al.*, 2012). According to Udayakumar *et al.* (2009), ashwagandha root and leaf extract demonstrated a hypoglycemic activity comparable to the standard drug glibenclamide in alloxan-induced diabetic rats. Ashwagandha has strong anti-oxidative properties which aid in the prevention of cellular damage caused by free radicals. In addition, ashwagandha has been shown to have anxiolytic, antidepressant, and neuroprotective effects in animal stress models because it improves memory, brain and nervous system functions (Singh *et al.*, 2011). However, there are few reports in the literature on the effect of ashwagandha about the impact of DM directly on brain and peripheral nerve damage.

- **Ashwagandha: Alternative Anti-diabetic Drug Sources:**

Ashwagandha (*Withania somnifera*, Family: Solanaceae), commonly called "Indian ginseng", is one of the most important herbs of Ayurveda "the traditional system of medicine in India" (Shivaraj *et al.*, 2021). The roots of ashwagandha are the primary part of the plant that is widely used as therapeutic agents and have the potential to benefit human health due to their high content of polyphenols and antioxidant activities (Sangwan *et al.*, 2008; Verma and Kumar, 2011). Ashwagandha roots are classified as rasayanas, which are thought to promote health and longevity by enhancing defense against disease, slowing the aging process, increasing an individual's ability to resist adverse environmental factors, and creating a sense of mental well-being (Wal *et al.*, 2019). Rasayanas are non-toxic Ayurvedic complex herbal preparations or individual herbs used to rejuvenate or attain the complete potential of an individual to prevent diseases and degenerative changes that lead to disease (Vayalil *et al.*, 2002). Ashwagandha is known for its medicinal properties which make it a viable therapeutic agent for many human illnesses such as epilepsy, depression, arthritis, diabetes, anxiety, cancer, microbial infection, immunomodulation, and neurodegenerative disorders. Moreover, ashwagandha has analgesic, rejuvenating, regenerating, and growth-promoting properties (Dar *et al.*, 2015; Dutta *et al.*, 2019).

Although synthetic oral hypoglycemic drugs alongside insulin are the main route for controlling DM, they fail to reverse the course of its complications completely and further worsen due to the fact that they also demonstrate prominent side effects (Saikat *et al.*, 2021). This forms the main force for discovering alternative sources of anti-diabetic agents. For a long time, diabetics have been treated orally with herbal medicines or extracts, because plant products are often thought to be less toxic and free of side effects than synthetic ones (Wadkar *et al.*, 2008). There is now a trend toward the use of complementary medicines to treat and decrease DM-related complications (Head, 2006). The anti-hyperglycemic effects resulting from treatment with plants are usually attributed to their ability to improve pancreatic tissue performance, which is done by increasing insulin secretions or by reducing the intestinal absorption of glucose (Kooti *et al.*, 2016).

- **Anti-diabetic Effects of Ashwagandha:**

Diabetes mellitus arises as a result of glucose homeostasis defects and it is

characterized by high blood glucose levels (hyperglycemia) resulting from defects in insulin secretion, action, or both (Mellitus, 2005). Alloxan is a cytotoxin that has been shown to cause chemical diabetes in a wide range of animal species by damaging insulin-secreting cells, therefore inducing hyperglycemia (Lenzen, 2008). Alloxan is not only toxic to pancreatic cells but also to the liver, with a mechanism of action that includes sulfhydryl group attack, chelating action, enzyme and metabolic modifications, changes in electrolyte membrane transport, increased lipid peroxidation, and decreased antioxidant defenses (Szkudelski *et al.*, 1998). The intravenous administration of alloxan in the quoted dose range is usually toxic, so intraperitoneal administration should be preferred because it is easier and better tolerated (Radenković *et al.*, 2016). Alloxan is well known to cause hyperglycemia, it is noticed that it can cause marked hypoglycemia in the first 48 hours after administration due to the release of preformed insulin from damaged β-cells (Chagas *et al.*, 2018).

Plants' anti-hyperglycemic effects are typically attributed to their ability to improve pancreatic tissue performance, which is accomplished by increasing insulin secretions or decreasing intestinal glucose absorption (Kooti *et al.*, 2016). Ashwagandha root and leaf extract demonstrated hypoglycemic activity comparable to the standard drug glibenclamide in alloxan-induced diabetic rats. Ashwagandha flavonoids and phenolic compounds might be responsible for its hypoglycemic effect (Udayakumar *et al.*, 2009). According to Babu *et al.*, 2013, flavonoids and phenolic compounds exert their anti-diabetic effects by regulating glucose metabolism in hepatocytes and increasing glucose absorption from skeletal muscles and fatty tissue.

- **Anti-oxidative Effects of Ashwagandha:**

One of the mechanisms involved in diabetic-induced neuronal damage is most likely oxidative stress (OS), a relative oxidant overload caused by increased free radical production and/or decreased antioxidants (Vincent *et al.*, 2004). The extent of OS is determined by the balance between ROS formation, such as superoxide anion ($O_2^{\cdot -}$), and the antioxidant defense system, which includes superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) (RG, 2005). Lipid peroxidation (LPO) is a well-known OS marker that may be responsible for cellular injury and the irreversible oxidative damage of membranes that occurs during DM (De M. Bandeira *et al.*,2013). Hyperglycemia increases the production of free radicals, which attack membrane lipids and initiate LPO, resulting in the formation of toxic products such as malondialdehyde (MDA), a terminal compound of LPO which is more cytotoxic and stable than ROS (Taso *et al.*, 2019). MDA can quickly combine with biomolecules and contributes to cellular disruption, including liver and pancreatic cells, thus, deregulating glucose metabolism. Furthermore, the increase in LPO might be a reflection of the decrease in antioxidant defense systems in diabetic rats (Barrera *et al.*, 2018).

According to Singh *et al.* (2011) ashwagandha has strong antioxidant properties, which aid in the prevention of cellular damage caused by free radicals. Ashwagandha is known to modulate brain OS markers, such as SOD, CAT, GSH, and LPO (John, 2014). The antioxidant activity was demonstrated by glycowithanolides from ashwagandha by activating a dose-dependent increase in SOD, CAT, and GPx activity in the cortex and striatum of rat brains (Mandlik and Namdeo, 2021). This is in agreement with Anwer *et al.* (2012) and Tekula *et al.* (2018) who found that administration of ashwagandha caused a significant decrease in MDA, accompanied by a significant increase in the antioxidant level. The antioxidant properties of ashwagandha may be attributed to its high content of phenolic compounds (Alam *et al.*, 2011). Similar findings of Filipiak-Szok *et al.* (2017) confirmed the presence of caffeic acid, chlorogenic acid, and p-

hydroxybenzoic acids in dried powdered ashwagandha. Alam *et al.* (2011) reported that benzoic acid was detected in ashwagandha root extract, however, syringic acid was not. Also, Abdel-Wahhab *et al.* (2019) reported the presence of caffeic acid in ashwagandha root extract. Furthermore, Khalil *et al.* (2021) noticed that ashwagandha is rich in several phenolic acids such as syringic acid, benzoic acid, gallic acid, vanillic acid, and, p-coumaric acid. This agreed with Mukherjee *et al.* (2021) who confirmed that ashwagandha phytochemicals showed various pharmacological activities like neuroprotective, anti-aging, anti-stress/adaptogenic and anti-diabetic effects.

- **Anti-inflammatory Effects of Ashwagandha:**

Diabetes mellitus is widely regarded as an inflammatory disease, in which different cytokines concentrations have been found to rise in the serum of diabetic patients (Deng and Chow, 2010). According to Kong *et al.*, 2021, OS and neuro-inflammation have been identified as key pathophysiological triggers in a variety of diabetes-related microvascular complications, including DN. In the presence of inflammation, the ROS response is activated and contributed to the inflammatory response (Park *et al.*, 2015). ROS are considered strong stimuli for the release of pro-inflammatory cytokines including TNF-α, IL-1β, IL-6 and IL-1β itself can trigger signaling cascades resulting in excessive ROS (Murphy *et al.*, 2016). The pro-inflammatory cytokines are primarily produced by activated macrophages, play a role in the regulation of inflammatory responses and there is also evidence that they have pleiotropic effects on glia and neuron homeostasis in the central, peripheral, and autonomic nervous systems (Vallejo *et al.*, 2010).

According to Saleem *et al.* (2020), withanolides, the principal bioactive components of ashwagandha, are mainly found in roots and have neuroprotective, anti-inflammatory and anti-depressant properties. This agrees with Padigaru *et al.* (2020) who found that serum levels of inflammatory cytokines (TNF-α, IL-1β, and IL-6) were significantly decreased after treatment with ashwagandha, when compared to arthritic rats. In another study, the reduction in the serum level, protein, and mRNA expression of TNFα, IL-1β, and IL-6 was observed with ashwagandha supplementation in the high-fat diet plus extract group when compared with the high-fat diet group (Kaur and Kaur, 2017).

- **Effects of Ashwagandha on Peripheral Nerve Injury:**

Diabetes mellitus (DM) is a major cause of nerve damage, especially in the longer peripheral nerves that innervate the lower limbs (Zochodne, 2007). In fact, diabetic neuropathy (DN) is the most commonly diagnosed complication of DM affecting 30% of people with DM overall and > 50% of people over the age of 50 and is associated with both vascular and non-vascular abnormalities (Gheith *et al.*, 2016; Mezil and Abed, 2021). Although DN has the highest lifetime risk of any DM complication, it is one of the least studied DM complications because it is difficult to be measured directly and accurately, so treatment is solely focused on prevention through glucose control and pain and symptom management (Fisher *et al.*, 2019). DN can affect the nervous system in four ways, including peripheral symmetric neuropathy, autonomic neuropathy, proximal neuropathy, and focal neuropathy (Vinik *et al.*, 2013).

Peripheral symmetric neuropathy is the most prevalent type of DN and it is often associated with the feet and legs, but it can also affect the arms and hands (Gylfadottir *et al.*, 2019). The symptoms of peripheral diabetic neuropathy (PDN) include numbness (which may become permanent), pain, tingling, burning sensations (especially in the evening), muscle tone loss in the hands and feet, inability to sense heat, cold, or physical injury, and loss of balance (Tesfaye, 2013). In fact, PDN that affects the feet can make standing and walking difficult which

increases the risk of falling (Said, 2007). According to Kaloyanova (2021), chronic hyperglycemia is closely linked to degenerative abnormalities in the PNS due to ROS production. Indeed, OS contributes to the development of PDN by activating various inflammatory mediators, resulting in lipid membrane destruction and tissue injury. Previous studies reported that ashwagandha is known to have anti-inflammatory properties by preventing the initiation of adverse processes of sciatic nerve morphology, and internal cell functions leading to improved coordination, behavioral and physiological functions in alloxan-induced diabetic rats (Samadi Noshahr *et al.*, 2015; Sikandan *et al.*, 2018). It seemed that ashwagandha ameliorated the histopathological changes of sciatic nerve induced in diabetic rats and acted as an anti-inflammatory agent by significantly reducing the release of pro-inflammatory cytokines, such as TNFα, IL-1β, and IL-6 which are important in mediating its antinociceptive effects on peripheral neuropathic pain.

CONCLUSION

We can conclude that ashwagandha may have therapeutic benefits on some of the alloxan-induced changes, such as oxidative stress, inflammation, and peripheral nerve damage.

REFERENCES

Abdel-Hay, A., Sivaprasad, S., Subramanian, A., & Barbur, J. L. (2018). Acuity and colour vision changes post intravitreal dexamethasone implant injection in patients with diabetic macular oedema. *PloS one*, 13(6), e0199693.

Abdel-Wahhab, K., Mourad, H., Mannaa, F., Morsy, F., Hassan, L., and Taher, R. (2019). Role of ashwagandha methanolic extract in the regulation of thyroid profile in hypothyroidism modeled rats. *Molecular biology reports*, *46*, 3637-3649.

Alam, N., Hossain, M., Khalil, M., Moniruzzaman, M., Sulaiman, S., and Gan, S. (2011). High catechin concentrations detected in Withania somnifera (ashwagandha) by high performance liquid chromatography analysis. *BMC Complementary and Alternative Medicine*, *11*, 1-8.

Alam, N., Hossain, M., Khalil, M., Moniruzzaman, M., Sulaiman, S., and Gan, S. (2012). Recent advances in elucidating the biological properties of Withania somnifera and its potential role in health benefits. *Phytochemistry Reviews*, *11*, 97-112.

Anwer, T., Sharma, M., Pillai, K., and Khan, G. (2012). Protective effect of Withania somnifera against oxidative stress and pancreatic beta-cell damage in type 2 diabetic rats. *Acta poloniae pharmaceutica*, *69*, 1095-1101.

Babu, P., Liu, D., and Gilbert, E. (2013). Recent advances in understanding the anti-diabetic actions of dietary flavonoids. *The Journal of nutritional biochemistry*, *24*, 1777-1789.

Banerjee, M., Khursheed, R., Yadav, A. K., Singh, S. K., Gulati, M., Pandey, D., Prabhakar, P., Kumar, R., Porwal, O., Awasthi, A., Kumari, Y., Kaur, G., Ayinkamiye, C., Prashar, R., Mankotia, D., & Pandey, N. K. (2020). A Systematic Review on Synthetic Drugs and Phytopharmaceuticals Used to Manage Diabetes. *Current diabetes reviews*, *16*(4), 340–356.

Barrera, G., Pizzimenti, S., Daga, M., Dianzani, C., Arcaro, A., Cetrangolo, G., Giordano, G., Cucci, M., Graf, M., and Gentile, F. (2018). Lipid peroxidation-derived aldehydes, 4-hydroxynonenal and malondialdehyde in aging-related disorders. *Antioxidants*, *7*, 102.

Chagas, V., Coelho, R., Gaspar, R., da Silva, S., Mastrogiovanni, M., Mendonca, C., Ribeiro, M., Paes, A., and Trostchansky, A. (2018). Protective effects of a polyphenol-rich extract from Syzygium cumini (L.) skeels

leaf on oxidative stress-induced diabetic rats. *Oxidative medicine and cellular longevity, 2018*, 5386079. https://doi.org/10.1155/ 2018/5386079

Dar, N., Hamid, A. and Ahmad, M. (2015): Pharmacologic overview of Withania somnifera, the Indian Ginseng Cell. *Cellular and molecular life sciences, (72)*: 4445–4460.

De M. Bandeira, S., Da Fonseca, L., Da S. Guedes, G., Rabelo, L., Goulart, M., Vasconcelos, S. (2013). Oxidative Stress as an Underlying Contributor in the Development of Chronic Complications in Diabetes Mellitus. *International Journal of Molecular Sciences*; 14(2):3265-3284. https://doi.org/10.3390/ ijms 14023265.

Deng, R., and Chow, T. (2010). Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae Spirulina. *Cardiovascular therapeutics, 28*, e33-e45.

Dutta, R., Khalil, R., Green, R., Mohapatra, S. and Mohapatra, S. (2019): Withania somnifera (ashwagandha) and withaferin A: Potential in integrative oncology. *International Journal of Molecular Sciences,* 20(21): 5310-5315.

Filipiak-Szok, A., Kurzawa, M., Szłyk, E., Twarużek, M., Błajet-Kosicka, A., and Grajewski, J. (2017). Determination of mycotoxins, alkaloids, phytochemicals, antioxidants and cytotoxicity in Asiatic ginseng (Ashwagandha, Dong quai, Panax ginseng). *Chemical Papers, 71*, 1073-1082.

Fisher, L., Polonsky, W., and Hessler, D. (2019). Addressing diabetes distress in clinical care: a practical guide. *Diabetic Medicine, 36*, 803-812.

Gheith, O., Farouk, N., Nampoory, N., Halim, M.A., and Al-Otaibi, T. (2016). Diabetic kidney disease: world wide difference of prevalence and risk factors. *Journal of nephropharmacology, 5*, 49.

Gylfadottir, S., Weeracharoenkul, D., Andersen, S., Niruthisard, S., Suwanwalaikorn, S., and Jensen, T. (2019). Painful and non-painful diabetic polyneuropathy: clinical characteristics and diagnostic issues. *Journal of diabetes investigation, 10*, 1148-1157.

Head, K. (2006). Peripheral neuropathy: pathogenic mechanisms and alternative therapies. *Alternative medicine review, a journal of clinical therapeutic, 11*(4), 294–329.

John, J. (2014). Therapeutic potential of Withania somnifera: a report on phyto-pharmacological properties. *International Journal of Pharmaceutical sciences and research, 5*, 2131-2148.

Kaloyanova, K. (2021). Assessing the excitability changes of DRG neurons in models of diabetic neuropathy. The University of Sheffield, Faculty of Science, Biomedical Science. uk.bl.ethos. 837188.

Kaur, T., and Kaur, G. (2017). Withania somnifera as a potential candidate to ameliorate high fat diet-induced anxiety and neuroinflammation. *Journal of neuroinflammation, 14*, 1-18.

Khalil, H., Eliwa, H., El-Shiekh, R., Al-Mokaddem, A., Hassan, M., Tawfek, A., and El-Maadawy, W. (2021). Ashwagandha (Withania somnifera) root extract attenuates hepatic and cognitive deficits in thioacetamide-induced rat model of hepatic encephalopathy via induction of Nrf2/HO-1 and mitigation of NF-κB/MAPK signaling pathways. *Journal of Ethnopharmacology, 277*, 114141.

Kong, M., Xie, K., Lv, M., Li, J., Yao, J., Yan, K., Wu, X., Xu, Y., and Ye, D.

(2021). Anti-inflammatory phytochemicals for the treatment of diabetes and its complications: Lessons learned and future promise. *Biomedicine & Pharmacotherapy, 133*, 110975.

Kooti, W., Farokhipour, M., Asadzadeh, Z., Ashtary-Larky, D., and Asadi-Samani, M. (2016). The role of medicinal plants in the treatment of diabetes: a systematic review. *Electron Physician, 8*, 1832.

Lenzen, S. (2008). The mechanisms of alloxan-and streptozotocin-induced diabetes. *Diabetologia, 51*, 216-226.

Mandlik, D., and Namdeo, A. (2021). Pharmacological evaluation of Ashwagandha highlighting its healthcare claims, safety, and toxicity aspects. *Journal of Dietary Supplements, 18*, 183-226.

Martínez-Tellez, R., de Jesús Gómez-Villalobos, M., and Flores, G. (2005). Alteration in dendritic morphology of cortical neurons in rats with diabetes mellitus induced by streptozotocin. *Brain research, 1048*, 108-115.

Mellitus, D. (2005). Diagnosis and classification of diabetes mellitus. *Diabetes care, 28*, S5-S10.

Mezil, S., and Abed, B. (2021). Complication of diabetes mellitus. *Annals of the Romanian Society for Cell Biology*, 1546-1556.

Mukherjee, P., Banerjee, S., Biswas, S., Das, B., Kar, A., and Katiyar, C. (2021). Withania somnifera (L.) Dunal-Modern perspectives of an ancient Rasayana from Ayurveda. *Journal of Ethnopharmacology, 264*, 113157.

Murphy, A., Casey, A., Byrne, G., Chambers, G., and Howe, O. (2016). Silver nanoparticles induce pro-inflammatory gene expression and inflammasome activation in human monocytes. *Journal of Applied Toxicology, 36*, 1311-1320.

Ozougwu, J., Obimba, K., Belonwu, C., and Unakalamba, C. (2013). The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. *Academic journals, 4*, 46-57.

Padigaru, M., Morde, A., Muz, O., Orhan, C., Erten, F., Tuzcu, M., Ozercan, I., Sahin, K., and Rai, D. (2020). Efficacy of a Novel Integrated Active Herbal Formulation in Experimentally Induced Osteoarthritis Rat Model. *Current Developments in Nutrition, 4*, 1762-1762.

Park, J., Min, J., Kim, B., Chae, U., Yun, J., Choi, M., Kong, I., Chang, K., and Lee, D. (2015). Mitochondrial ROS govern the LPS-induced pro-inflammatory response in microglia cells by regulating MAPK and NF-κB pathways. *Neuroscience letters, 584*, 191-196.

Poznyak, A., Grechko, A., Poggio, P., Myasoedova, V., Alfieri, V., and Orekhov, A. (2020). The diabetes mellitus–atherosclerosis connection: The role of lipid and glucose metabolism and chronic inflammation. *International journal of molecular sciences, 21*, 1835.

Radenković, M., Stojanović, M., and Prostran, M. (2016). Experimental diabetes induced by alloxan and streptozotocin: The current state of the art. *Journal of pharmacological and toxicological methods, 78*, 13-31.

RG, A. (2005). Is there a balance between oxidative stress and antioxidant defense system during development. *Medical Journal of Islamic World Academy of Sciences, 15*, 55-63.

Said, G. (2007). Diabetic neuropathy—a review. *Nature clinical practice Neurology, 3*, 331-340.

Saikat, A., Hossain, R., Mina, F., Das, S., Khan, I., Mubarak, M., and Islam, M. (2021). Antidiabetic effect of

garlic. *Revista Brasileira de Farmacognosia*, 1-11.

Saleem, S., Muhammad, G., Hussain, M. A., Altaf, M., & Bukhari, S. N. A. (2020). Withania somnifera L.: Insights into the phytochemical profile, therapeutic potential, clinical trials, and future prospective. *Iranian Journal of Basic Medical Sciences,* 23(12), 1501.

Samadi Noshahr, Z., Shahraki, M. R., Ahmadvand, H., Nourabadi, D., & Nakhaei, A. (2015). Protective effects of Withania somnifera root on inflammatory markers and insulin resistance in fructose-fed rats. *Reports of biochemistry & molecular biology*, 3(2), 62–67.

Sangwan, R., Chaurasiya, N., Lal, P., Misra, L., Tuli, R., and Sangwan, N. (2008). Withanolide A is inherently de novo biosynthesized in roots of the medicinal plant Ashwagandha (Withania somnifera). *Physiologia plantarum, 133*, 278-287.

Shivaraj, Y., Kaveri, K., Asiya Nuzhat, F., and Rajesh, R. (2021). Acute oral toxicity study of "Polyherbal formulation (Rosmarinus officinals, Ashwaghandha and Amla) in Wistar rats". *Journal of Entomology and Zoology Studies,* 9(4): 16-27

Sikandan, A., Shinomiya, T., & Nagahara, Y. (2018). Ashwagandha root extract exerts anti-inflammatory effects in HaCaT cells by inhibiting the MAPK/NF-κB pathways and by regulating cytokines. *International journal of molecular medicine,* 42(1), 425–434. https:// doi. org/ 10.3892/ijmm.2018.3608

Singh, A., Kukreti, R., Saso, L., and Kukreti, S. (2019). Oxidative stress: a key modulator in neurodegenerative diseases. *Molecules (Basel, Switzerland), 24*, 1583.

Singh, N., Bhalla, M., de Jager, P., and Gilca, M. (2011). An overview on ashwagandha: a Rasayana (rejuvenator) of Ayurveda. *African Journal of Traditional, Complementary and Alternative Medicines: AJTCAM*, 8(5 Suppl), 208–213. https://doi.org/10.4314/ ajtcam.v8i5S.9.

Szkudelski, T., Kandulska, K., and Okulicz, M. (1998). Alloxan in vivo does not only exert deleterious effects. *Physiological research, 47*, 343-346.

Taso, O., Philippou, A., Moustogiannis, A., Zevolis, E., and Koutsilieris, M. (2019). Lipid peroxidation products and their role in neurodegenerative diseases. *The Annals of Research Hospitals*, Volume 3. doi: 10.21037/arh.2018.1

Tekula, S., Khurana, A., Anchi, P., and Godugu, C. (2018). Withaferin-A attenuates multiple low doses of Streptozotocin (MLD-STZ) induced type 1 diabetes. *Biomedicine & Pharmacotherapy, 106*, 1428-1440.

Tesfaye, S. (2013). Impact of painful diabetic polyneuropathy on patients. In Painful Diabetic Polyneuropathy (Springer), pp. 155-166.

Udayakumar, R., Kasthurirengan, S., Mariashibu, T.S., Rajesh, M., Anbazhagan, V., Kim, S., Ganapathi, A., and Choi, C. (2009). Hypoglycaemic and hypolipidaemic effects of Withania somnifera root and leaf extracts on alloxan-induced diabetic rats. *International journal of molecular sciences, 10*, 2367-2382.

Vallejo, R., Tilley, D., Vogel, L., and Benyamin, R. (2010). The role of glia and the immune system in the development and maintenance of neuropathic pain. *Pain practice, 10*, 167-184.

Vayalil, P., Kuttan, G., and Kuttan, R. (2002). Rasayanas: evidence for the concept of prevention of diseases. *The American journal of Chinese medicine, 30*, 155-171.

Verma, S., and Kumar, A. (2011). Therapeutic uses of Withania somnifera (Ashwagandha) with a note on withanolides and its pharmacological actions. *Asian Journal of Pharmaceutical and Clinical Research, 4*, 1-4.

Vincent, A., Russell, J., Low, P., and Feldman, E.L. (2004). Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocrine reviews, 25*, 612-628.

Vinik, A., Nevoret, M., Casellini, C., and Parson, H. (2013). Diabetic neuropathy. *Endocrinology and Metabolism Clinics, 42*, 747-787.

Visweswari, G., Christopher, R., and Rajendra, W. (2013). Phytochemical screening of active secondary metabolites present in Withania somnifera root: role in traditional medicine. *International journal of pharmaceutical sciences and research, 4*, 2770.

Wadkar, K., Magdum, C., Patil, S., and Naikwade, N. (2008). Antidiabetic potential and Indian medicinal plants. *Journal of herbal medicine and toxicology, 2*, 45-50.

Wal, A., Wal, P., Rai, A., Tiwari, R., and Prajapati, S. (2019). Adaptogens with a Special Emphasis on Withania Somnifera and Rhodiola Rosea. In Nutrition and Enhanced Sports Performance (Elsevier), pp. 407-418.

Wang, G., Li, G., Wang, Y., Yang, T., and Ouyang, Y. (2001). Characterization of neuronal cell death in normal and diabetic rats following exprimental focal cerebral ischemia. *Life Sciences, 69*, 2801-2810.

Zochodne, D. (2007). Diabetes mellitus and the peripheral nervous system: manifestations and mechanisms. Muscle & Nerve: *Official Journal of the American Association of Electrodiagnostic Medicine, 36*, 144-166.