



A COMPREHENSIVE REVIEW ON PHYTOCHEMICALS OF *WITHANIA SOMNIFERA* AND ITS ROOTS EFFECT ON MUSCLE RECOVERY AND HORMONE LEVELS

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Abstract: In Ayurveda, several herbal supplementations are described for muscle strengthening and muscle recovery. In the Indian traditional system, Ayurveda *Withania somnifera* (Ashwagandha) is said to have health-promoting effects, and some of the constituents can manage muscle repair and enhance activity. Ashwagandha roots can reduce the muscle recovery time and elevate the levels of testosterone which further increases muscle mass. Ashwagandha supplementation is efficacious in boosting stamina in athletes and beneficial over synthetically prepared supplements. Lately, an era of anabolic steroids has taken over the market despite knowing it leads to a decrease in endogenous testosterone production with several side effects. In comparison to ashwagandha, ashwagandha has the upper hand on anabolic steroids subjected to build muscle strength since it has fewer side effects. Ultimately Ashwagandha appears to be a sports medicine with positive effects on muscle strength, endurance, and recovery from damage caused by intense training in athletes and bodybuilders. In addition, ashwagandha decreases cortisol levels, lowering body fat, which proved to be a boon to bodybuilders. The current review covers the actual competence of *Withania somnifera* and its profound effect on muscles, body fat, and testosterone levels.

IndexTerms - *Withania somnifera*, withanolides, muscle recovery, creatine kinase, testosterone, cortisol.

INTRODUCTION

Ashwagandha is a crucial drug in the *Rasayana* and *bajikarana Chikitsa* branch of Ayurveda which focuses on the comparative increase in the endurance and immunity of a person. Earlier a medical practitioner named Charaka (200 BC and 200 AD) specified this plant in the oldest text in Ayurveda called *Charaka Samhita*. Mixtures of several parts of ashwagandha are used for the treatment of diseases related to the rheumatic, skin, and respiratory systems^{1,2}.

Most of the population in the world is taking an interest in natural products due to their satisfying effects on health care. Nutraceuticals are substantial preparations that rely on traditional knowledge and are compound forms of preparations derived from ancient Ayurveda systems with excessive activity and stability. *Withania somnifera* is stated in several Asian therapies for some diseases and disorders. Ashwagandha is observed as an excellent health promoter and has a major property to relieve stress³. Ashwagandha is formulated in many products in the Indian market with its specific therapeutic usage. These products are processed by traditional Ayurvedic preparations and also by modern formulation. The Ayurvedic formulation includes *vati*, *ghritas*, *avleha*, *pills*, and *powders*. Modern formulations are tablets that contain ashwagandha powder, capsules, and extracts that are available in the market⁴.

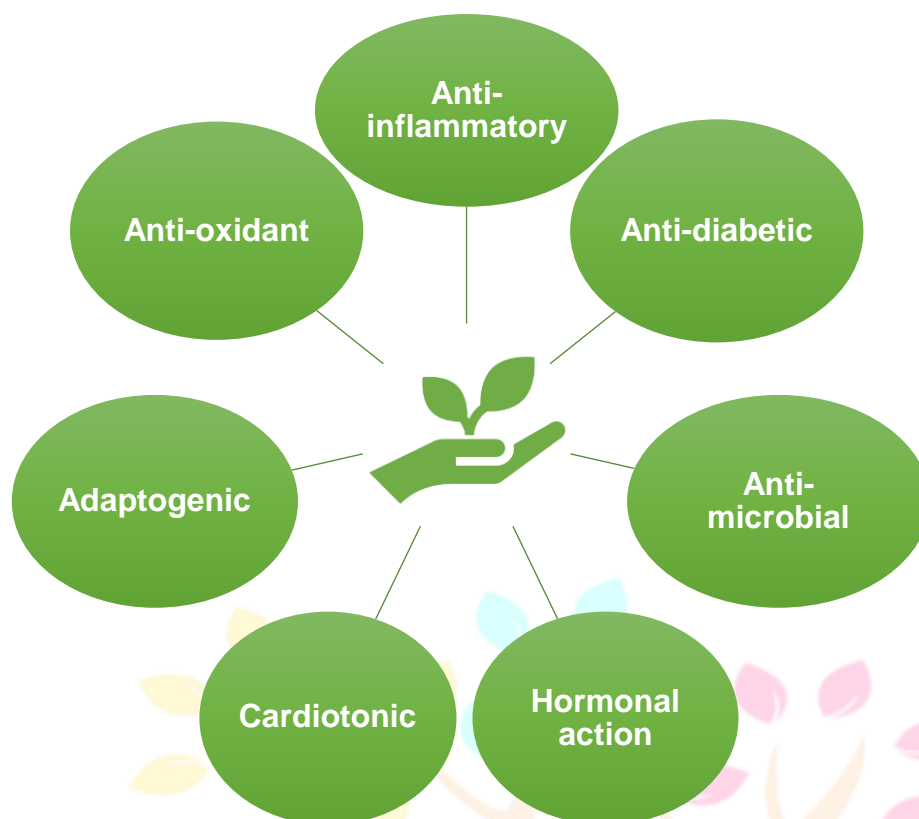


Figure 1. Numerous health benefits of Ashwagandha

Ashwagandha (*Withania somnifera*) is also known as winter cherry or Indian ginseng. Ashwagandha means ‘smells like a horse’⁵. This plant is approximately 4-5 feet tall. In Ayurveda, Siddha, and Unani there are around 100 formulations of this plant. Withania extract has relatively equal activity compared to *Panax ginseng* but has superiority over *panax ginseng* because it does not lead to ginseng-abuse syndrome (a condition occurred due to increase in blood pressure, edema, muscle tension, and insomnia)^{4,6}.

Withania somnifera belongs to the *Solanaceae* family which is also to be known as the deadly nightshade family⁷. There are a total of 26 species of which only 2 that is *W. somnifera* and *W. coagulans* (also called Paneer ke phool, punir dodi, and paneer bandh) both are economical and have considerable medicinal importance in many regions. Other than these Remaining important species are *W. simonii*, *W. adunensis*, and *W. riebeckii*⁸. Biological classification of Withania is it belongs to Plantae (kingdom), Tracheophytes (subkingdom), Angiospermae (division), Eudicots (Class), Solanales (Order), *Solanaceae* (Family), *Solanoideae* (Sub-family), *Physaleae* (Tribe), *Withania* (Genus), *Somnifera*, *Coagulans*, *Adpressa*, *Frutescens* (Species)^{8,9}.

Biological species (Withania) available throughout the world:

- ***Withania Somnifera*:** immensely obtainable in areas of India, Europe, Australia, and Africa^{8,9}.
- ***Withania adpressa*:** In Morocco, India, North Africa, Algeria, Mediterranean Basin¹⁰.
- ***Withania coagulans*:** East India, Iran, Pakistan, Afghanistan, widen from North Africa to South Asia and also in the Mediterranean region^{11,12}.
- ***Withania Frutescens*:** Southern Europe, Northern Africa, Western and Southern part of Asia¹³.



Figure 2. Captured images of major parts of ashwagandha

Table 1. Biological Description of Ashwagandha^{8,9}

Parts	Description
Leaves	The average length of the leaves is about 10-15 Cm. Its leaves are relevantly dull greenish in color, oblique, Elliptical, Petiolate, Simple, Shiny, and Smooth, Leaves are equivalent on the vegetative shoots and on floral shoots leaves are opposite. The above portion is almost hairless but the below leaf is crowded with hairs.
Roots	Roots are Brownish-white or light yellow and in length are extended from 15-25 Cm. Additionally, it Smells like a Horse and its taste is Bitter. It is a long tap root, Stout and fleshy.
Flowers	Flowers are in diameter of 4-6 mm. They are Inconspicuous, solitary, bisexual, and pedicellate. Corolla is greenish-yellow and 5 stamens. Anthers are ditheous and basifixed. Ovaries present in it are bilocular.
Fruits	This plant grows Orange-red berries.
Branches	Branches are enclosed with trichomes and are Round in shape

Bioactive constituents in *Withania somnifera*:

W.Somnifera is embedded with many paramount phytochemicals that assist against pathogens by acting as an antimicrobial. Constituents such as secondary metabolites change with variations in the type of tissue, growth conditions, and species of the genus *Withania*. The above variations have influence on the unpredictable medication and health-promoting factors of several commercial preparations available in the market for this species of plant.

The records state that there are 62 primary and secondary metabolites (major and minor) in the leaves and roots hold about 48 among them 29 are available in both leaves and roots¹⁴.

Table 1. Bioactive compounds available in different biological species (Withania) and their suitable uses^{7,9-14}.

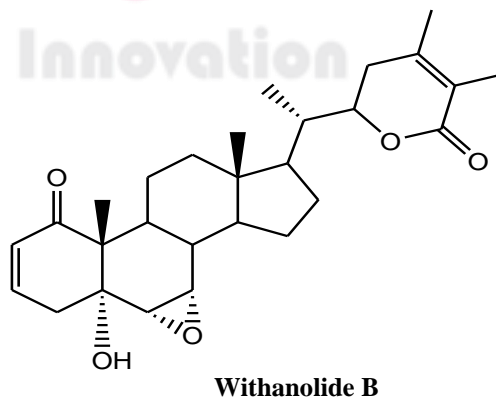
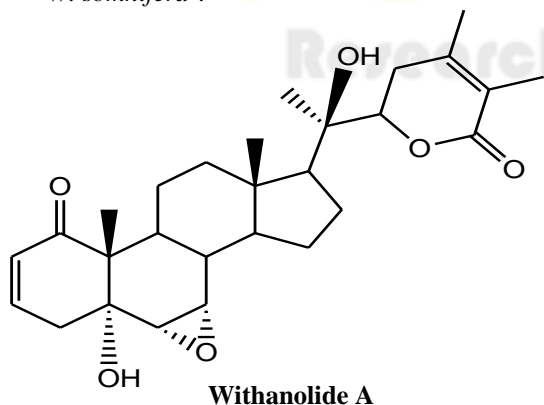
Species	Bioactive compounds	Uses
<i>Withania Somnifera</i>	Alkaloids, phenols, flavonoids, saponin, tannins, Steroidal lactones, sitoindosides, somniferiene, somniferinine, pseudotropine, scopoletin, anaferine, cysteine, withanine, withaferin, withananine, withanolides, tropanol, anahygrine, chlorogenic acid.	Anti-inflammatory, antistress, reproductive, antistress, impotence, antidiabetic, antimicrobial, anticancer.
<i>Withania coagulans</i>	Withanolides, coagulans, coagulanolides, coagulins, anthocyanins, carbohydrates, fats, protein, fiber, amino acids, fatty acids, tannins, flavonoids, phenols, minerals, etc.	Antidiabetic, antioxidant, antifungal, anti-inflammatory, anticancer, neuroprotective, anti-cytotoxic, hypolipidemic, and antihelminthic.
<i>Withania adpressa</i>	Coagulin L, nicotiflorin, wadpresin, withanolides f, and J.	Immunomodulatory, antiproliferative.
<i>Withania frutescens</i>	Terpenoids, mucilage, tannins, flavonoids, polyphenols, saponins.	Antibacterial, antidiabetic, antifungal, anti-inflammatory, and used for wound healing.
<i>Withania aristata</i>	Withaferin A, withanolides A, withastramonolide withanone, 27-hydroxywithanone, physagulin.	Analgesic, anticancer, anti-inflammatory, anticholinesterase, antioxidant.

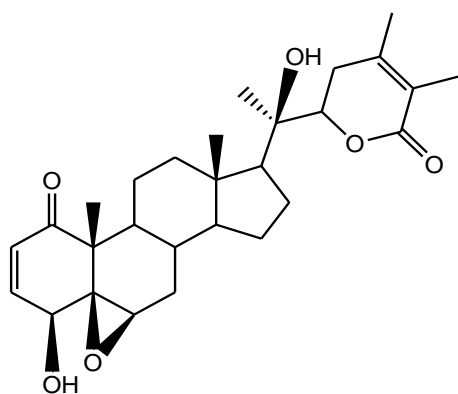
There are about twelve alkaloids, forty withanolides, and different sitoindosides isolated from this herb¹⁵. Numerous amino acids, flavonoids, glycosides, tyrosine, alanine, glucose, glutamic acid, glycine, cysteine, chlorogenic acid, iron, condensed tannins, and aspartic acid also have been extracted¹⁴.

Withanolides

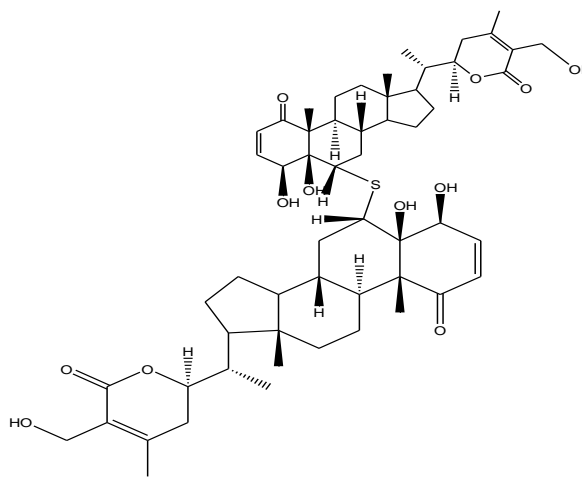
Withanolides is a precursor hormone and can modify itself in human physiology whenever it is necessary⁸. Generally, withanolides are considered to be significant classes of steroidal lactones. There are 15 genera of family Solanaceae identified which contain nearly 130 withanolides. Apart from this, some other families such as Leguminosae and Labiatae have been claimed to isolate withanolides from it. Some marine organisms are also capable of isolating withanolides. The plant *Withania Somnifera* assembles a substantial amount of withanolides involving varied functional groups. Withanolides are lead biochemicals with a proportion from 0.001-0.5% dry weight concentration in the leaves and roots of *Withania somnifera*. Withanolides are natural steroidal lactone containing 28-carbon constructed on an ergostane skeleton. The C-22 and C-26 carbon atoms are oxidized resulting in five-membered lactone rings. Withanolides are also defined as 22-hydroxy ergostane-26-oic acid-26,22-lactone. The major factor is that the C-8 or C-9 side chains show a lactone or lactol ring. As there are variations in the lactone ring it may be either five- or six-membered ring and might also be fused with carbocyclic moiety of the molecule through a C-C bond or an O-bridge.

Modification to the carbocyclic backbone or side chains caused by the structural derivatives of withanolides, includes withanolides A and D, withaferin A, and withanone. These compounds are polyoxygenated and contain a steroidal nucleus in which all C atoms oxidize. Ashwagandhanolide which is a new dimeric thiowithanolide is been isolated from the roots of *W. somnifera*⁹.





Withanolide D



Ashwaghandhanolide

Biosynthesis of withanolides

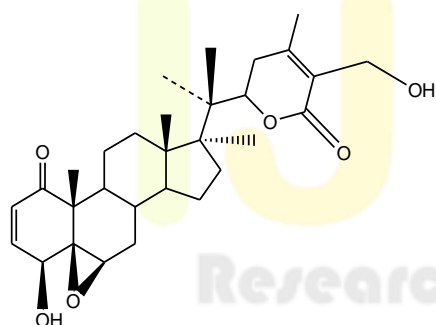
As yet there are not many recommendations on the clarification of the development of withanolides in ashwagandha, however, there are 2 routes that have been described as cytosolic mevalonate and 2-C-methyl-D-erythritol-4-phosphate (DOXP/MEP) pathway¹⁶.

The biosynthesis begins with the acetyl co-enzyme A activity to form cholesterol. Acetyl co-A is then merged and combined into two different mevalonic acid from which the R-type is utilized in triterpene biosynthesis whereas the S-type is inert. Since a carbon atom undergoes reduction, the R-type is modified to IPP (isopentenyl pyrophosphate) that on condensation with its own that is isomer 3,3-dimethyl allyl pyrophosphate (DMAPP) forms GPP (geranyl pyrophosphate). Further by continuing condensation FPP (farnesyl pyrophosphate) is formed. Furthermore, condensation of 2 molecules of FPP forms squalene in the presence of enzymes NADPH and squalene synthase. Later on, squalene on oxidation forms squalene 2,3-epoxide undergoing ring closure to generate lanosterol. The 24-methylene cholesterol formation has not yet been elucidated and till date it is not assured that it is precursor of lactones or not. Observation states that at C-22 hydroxylation and δ -lactonization occurred. In between position C-22 and C-26 of 24-methylene cholesterol are considered part of the withanolides pathway¹⁴.

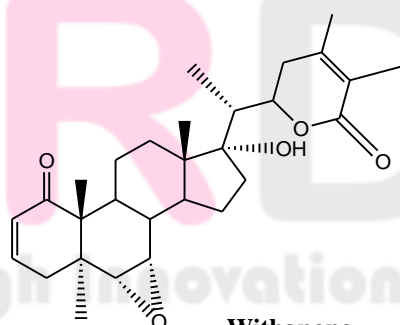
Withaferins

Withaferin A also named as 4 β ,27-dihydroxy-1-oxo-5 β , 6 β -epoxywitha-2-24-dienolide. A successful Isolation of first withaferin A achieved from the leaf section of South Asian *Withania Somnifera* with a concentration range of 0.13-0.31% dry weight. Statistically, the Indian chemotypes of *withania somnifera* are analyzed by TLC densitometry which states that leaves are the only part of the whole plant that contain withaferin A. The concentration of withaferin A in leaves is about 1.6%¹⁷.

Withaferin A has extensive activity as it is utilized for the medication for ulcers and carbuncles. It also exhibits radio-sensitizing effects on cancer and consumed for the treatment of tumors. Also, withaferin A is efficient in promoting immunosuppressive action on two lymphocytes B and T. Some other activities are observed such as antibacterial, anti-arthritic, immunomodulating, and antitumoral¹⁸. The motive behind Withaferin A has antibiotic activity is it contains an unsaturated lactone ring¹⁹. Withaferin A as well as withanone both are angiogenesis inhibitors and both show anticancer activity¹⁴.



Withaferin A



Withanone

Withanamides

Fruits of *W.Somnifera* consist of methanolic extract from which withanamides A are purified. The basic structure contains a 5-hydroxy tryptamine (serotonin) along with hydroxy fatty amide and diglucosidic moieties. The structural difference between withanamide A and withanamide C is in their side chain of the fatty acid. withanamide A has 2 double bonds in its side chain which becomes the only difference from withanamide C. It is been detected that withanamide A and C assist in neutralizing toxicity caused by β -amyloid protein (BAP) and provide protection to cells from causing death of cells. Withanamide A has a prominent role in the therapy of Alzheimer's disease. As it has the potential to stop lipid peroxidation (LPO) and consequently shows antioxidant properties. Withanamide A has selective COX-1 and COX-2 (cyclooxygenase) enzymes therefore it also suppresses tumor cell reproduction^{20,21}.

Sitoindosides

Sitoindoside is obtained from *W.Somnifera* plants which regularly contain a glucose molecule at the C-27 position. Two acyl glucosides sitoindoside VII & VIII, and two glycowithanoloids, sitoindoside IX and X are the phytochemicals that possess

the anti-stress activity and aid to regain the memory. These are the phytochemicals that exist in the roots of the plant and are isolated from it^{15,22}.

Alkaloids

Several alkaloids such as somniferinine, somniferin, somine, nicotine, anhygrine, cuscohygrine, withanine, withaninine, withaninine, pseudo-withanine, tropine, anaferrine, choline, and 3 α -triglyoxytropine. These alkaloids are responsible for the sedative and hypotensive actions. Additionally, studies reported that overall alkaloid present in the root ranges between 0.13%-0.31% and may approach 4.3% which is proclaimed according to various regions or countries, On the other hand, there are five such undefined alkaloids present in the leaves which yields nearly 0.09%. The range of yield varies depending on particular aspects such as techniques of isolation, ecological factors, or variations in the genetic constitution²³.

Other phytoconstituents

The roots contain glycosides, withanilic, starch, reducing sugar, peroxidases, dulcitol, hentriacontane, and various amino acids which involve alanine, cysteine, glutamic acid, aspartic acid, glycine, and tyrosine. Where leaves hold chlorogenic acid, flavonoids, withanone, and calystegines. It occurs that a sufficient quantity of tannin is present in the fruits^{14,23}.

PHYSICAL PERFORMANCE IN MALES

Previously some researchers revealed that in their randomized, prospective, double-blind, placebo-controlled studies they estimated the effect of ashwagandha roots powdered supplementation on muscle strength and muscle recovery. Subjects were selected fulfilling the eligibility criteria for the study. Accordingly, 57 subjects were eligible out of which 50 accomplished the study split up into 25 of the ashwagandha group and 25 of the placebo group. Participants in the ashwagandha group were prescribed to take two 300mg capsules of ashwagandha root extract (KSM-66) for 8 weeks.

The National Strength and Conditioning Association (NSCA) managed the resistance training designed for producing intense damage to muscles consisting of exercises in sets for upper as well as lower body muscles. Each Participant of both groups was informed to attend the training session every single day including that a rest day in a week was planned. Practically participants were guided efficiently and safely in weight lifting. They were commanded to reach maximum reps till the failure occurred to achieve excessive muscle damage. Respective weekly transition in the projected exercise program. The vital role-playing exercises were leg extension, leg curl, and barbell squats for the lower body and for the upper body as bicep curl, cable row, chest press, dumbbell shoulder press, and triceps push down. The repetition plan was selected as an alternative change until the last week of the session²⁴.

As per research on male sprinters, the effect of ashwagandha was analyzed, and the data was collected by t-test. A serious difference was observed in the control and the ashwagandha group in physical activities such as standing broad jumps, 50-yard dash, sit-ups, pull-ups, running or walking for 12 minutes, and shuttle runs. Ultimately overseeing the group consuming ashwagandha has comparatively greater strength in all formats with negligible side effects. They concluded the studies recommend that consuming ashwagandha in other sports that need Immense strength, core strength, and coordination is also beneficial for players²⁵.

A single-blinded technique is used on a group of thirty-two male Hockey players. Groups were divided into the ashwagandha group (n=16) and the placebo group (n=16). In capsules 500mg ashwagandha roots to the ashwagandha group and 500mg sugar powder to the placebo group was prescribed by the researchers as 1 capsule/day taken with milk after the meal for 8 weeks. The experiment was characterized by core muscle strength and stability which was analyzed by a t-test assessed by SPSS-20 (Statistical Package for Social Sciences) software. They reported that ashwagandha elevates stamina, instantaneous recovery from stress and fatigue developed due to resistance training, and may also assist in improving sufficient lean muscle. Scientifically they stated that the use of ashwagandha supplements has outstanding benefits for athletic sportsmen^{26,27}.

Another randomized, prospective, double-blind, placebo-controlled technique reported the effect of the ashwagandha root extract on cyclists. A total of 40 candidates were assigned and two Groups the ashwagandha group (n=20) and the placebo group (n=20) were designed. Two capsules of 500mg ashwagandha a day for 8 weeks were prescribed. Candidates were tested before and after the training program. The training was performed by using the FitNex 200 treadmill due to certain considerations. There were no signs of such adverse events in the study. The endpoint reported was, that there was an improvement in the performance of the cyclists assigned in the ashwagandha group^{27,28}.

Circumstances of muscle recovery

The damaged skeletal muscle tissue is caused by intense resistance training associated with muscle damage. Similarly, certain damage to the muscle gives rise to a decrease in the motor activity by slowing the movements resulting in persistently minimal growth from the training session.

Mechanism of muscle recovery

Straightly muscle recovery is a frequent decrease in time on account of damage caused by intense training. Prominently creatine kinase is referred to as a protein collected from the blood and examined as essential for muscle tissue. Measuring creatine kinase levels aids in defining muscle damage. While immense exertion of muscle, the muscles are necrotized leading to damage to the muscle filaments and resulting in the release of such soluble proteins as creatine kinase leaking from the muscle tissue throughout the bloodstream. Naturally, it takes 1-10 days to restore the damaged muscle and to reach creatine kinase to its ground state. At the starting to get the training session habitual, short-term exercises were programmed which can manage low damage to the tissue as adaptation and strengthening muscles are the reasons behind it. Serum creatine kinase was frequently measured in the timeline of 24 hours and 48 hours after completing the first training session as well as 24 hours and 48 hours after finishing the last training session almost 8 weeks later. It states that recovery level from damage correspondingly depends on the rise of serum creatine kinase approximately from hour 24 to hour 48 after the session. Even a moderate elevation in muscle protein levels in the blood leads to profound repair of tissue.

Several mechanisms are involved in muscle recovery and have certain properties that are responsible for the synergistic effects like anti-inflammatory, analgesic, and decrease in the lactic acid as well as nitrogen urea level in blood. Along with antioxidant action for free radical damage to the central nervous system and muscle. Commonly in such veins muscle soreness is usual in those candidates who are not frequently exercising. In the timeline of 24 and 48 hours after training the

delayed onset muscle soreness (DOMS) appears and the soreness leads to depressing muscle activity which affects exercise. As a result, Ashwagandha has a counter impact on DOMS due to its positive effect on muscle damage²⁴.

Effects on serum testosterone

In a randomized control trial, those males who consumed ashwagandha roots consistently observed an increase in their testosterone concentration, precisely males ($n=57$) aged 18-50 years had changes in their testosterone levels with an increasing order from 630.5 ± 231.9 to 726.6 ± 171.6 ng/dl, ($p < 0.001$) was observed. Comparatively, the placebo group did not observe such changes in testosterone while those who took ashwagandha in the ashwagandha group had observed changes^{24,29}.

In the studies, a randomized control trial of men ($n=19$) and women ($n=11$) 240 mg ashwagandha was consumed regularly for about 60 days. It was recorded that the elevation in the total testosterone levels was from 472.9 ± 45.0 to 526.9 ± 48.0 ng/dl ($p = 0.038$). In women, there was not a remarkable change in testosterone. Most importantly, the testosterone levels of the exposure group were not statistically significant compared to the placebo group ($p = 0.158$)³⁰.

Effects on cortisol level

Generally, cortisol is a typical stress hormone secreted by the adrenal gland and ultimately is responsible for the stress. Cortisol is secreted also if the levels of blood sugar are very low. According to research, ashwagandha has the ability to decrease cortisol levels and gives rise to its anti-stress activity. There was a moderate adverse effect observed but there was not a single side effect occurred which recommends a high concentration of full-spectrum ashwagandha root extract has an efficient effect on a person's life quality and resistance toward stress. Ashwagandha can also overcome stress-related infertility by increasing the levels of antioxidants. It also assists in conditions like depression.

Studies stated that 64 stressed adults were observed for 60 days after consuming 600 mg of high-concentration ashwagandha extract daily. In the end, it was concluded that about 79% reduction in the severe depression and with no adverse effect.

Studies have reported the antistress effect of ashwagandha along with reducing anxiety and fatigue. An increase in cortisol levels leads to stress. Ashwagandha has the potential to reduce cortisol levels in the blood. Classically this herb has been prominently used to treat erectile dysfunction, weakness, minor sexual desire in both men and women, and anxiety in men. In men this herb has a tremendous impact on the hormones as it elevates testosterone levels and luteinizing hormone (LH), decreasing follicle-stimulating hormone (FSH)³¹.

Growth of *W.Somnifera* as a dietary supplement

As per the FDA (Food and Drug Administration) products of *W.Somnifera* fall under the category of "botanical dietary supplements". To date, plenty of products are been formulated and are supplied to Indian citizens. National Institutes of Health Office of Dietary Supplements reported that extreme growth in the consumption of WS products and more than 1300 commercial products are available in the US market.

Year the incredible growth in the selling of *W.Somnifera* led it as a top-selling herb in the United States. Accordingly, it rose from the 8th position in the year 2016 to reach the 5th most sold herb in 2019 with sales of more than \$10 million from mainstream sources (drug stores and grocery stores) and in case of natural channels (supplements and specialized retail store), it was not less than \$13 million³¹.

SUMMARY:

There are many medicinal benefits of roots of ashwagandha plant and hence, used as a nutraceutical which is growing day by day in the market. Its action on the muscle assist to reduce muscle recovery time due to its anti-stress and anti-inflammatory activity. This may help the athletes to overcome from muscle stress caused by intense training. The major components in ashwagandha are group of steroidal lactones and some other phytoconstituent mentioned in the article. By increasing levels of testosterone in the body it also boosts energy. While, reducing cortisol secretion in the body helps to manage stress.

CONCLUSION:

Consumption of ashwagandha roots as a supplement gives numerous health benefits. Ashwagandha possess some effects like anti-diabetic, anti-microbial, adaptogenic, cardiogenic, etc. Apart from this ashwagandha is also useful as a sports medicine. Professional athletes were found to experience its effect to reduce stress and elevate testosterone in them. Important constituents such as steroidal lactone, alkaloids present in ashwagandha holds major activities. Mixtures of several parts of ashwagandha are used for the treatment of diseases related to the rheumatic, skin, and respiratory systems. Roots of ashwagandha can reduce the time for muscle recovery from damage cause to muscle on intense exercise.

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