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Phytochemical Extraction and Characterization of Roots of *Withania Somnifera* for Its Anti-Bacterial, Anti-Oxidant, Anti-Inflammation and Analgesic Activity

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Abstract:
Medicinal plants are a source of naturally active compounds used extensively by tribal people worldwide for many ailments. Withania somnifera (WS) is one such plant used to treat many ailments from the time of Ayurveda. The dried roots of Withania somnifera are widely used in the treatment of many disorders, the current investigation aimed at extraction and detection or screening of active phytochemical compounds from different extracts of Withania somnifera root. From chemistry point of view, the drug contains group of biologically active constituents known as withanolides. The chemical structures of withanolides have been studied and they are widely distributed in family Solanacae. Phytochemical screening of different extractions revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, and glycosides which could account for its varied medicinal properties like anti-inflammatory, anti-oxidant, anti-analgesic. The root samples of Withania somnifera are used to examine the anti-bacterial activity against some pathogenic bacteria such as Escherichia coli and Bacillus subtilis. The anti-bacterial activity of the powder extract was done with Chloroform, methanol and petroleum ether. Petroleum ether extract showed minimum anti-bacterial activity followed by aqueous, chloroform and methanolic extract. In the present study, the free radical scavenging potential of three extracts of the root of Withania somnifera was assessed by measuring its capability for scavenging 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Our study demonstrated that the three different extracts of Withania somnifera root showed different level of antioxidant activity and is a potential source of antioxidants and thus could prevent many radical related diseases. In the present study, withaferin A (active component of Withania somnifera), a steroid lactone was examined for its analgesic and anti-inflammatory properties employing different experimental models in mice. The study was done to evaluate the analgesic and anti-inflammatory activity of the aqueous, methanolic, choloroform and petroleum ether extract of the roots of Withania somnifera . The analgesic activity was studied using Eddy's Hot Plate method in swiss albino mice and for anti-inflammatory investigation Carrageenan-Induced Paw Edema method was applied.

Keywords*: Phytochemical Extraction, Withania Somnifera, Anti-Bacterial, Anti-Oxidant, Anti-Inflammation, Analgesic Activity.*

CHATPER – I

1. Introduction

1.1. Herbal Medicine and Its Importance

Plants are a source of large amount of drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, antimicrobials etc. A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. Plants have been known to relieve various diseases in Ayurveda. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda. Extraction of the bioactive plant constituents has always been a challenging task for the researchers.

1.2. Withania Somnifera

1.2.1. Introduction

Ashwagandha (*Withania somnifera*), also known as Indian ginseng, and as Indian Winter Cherry is an important ancient plant, the roots of which have been employed in Indian traditional systems of medicine, Ayurveda and Unani. It grows in dry parts in sub-tropical regions. Rajasthan, Punjab, Haryana, Uttar Pradesh, Gujarat, Maharashtra and Madhya Pradesh are the major Ashwagandha producing states of the country. The estimated production of Ashwagandha roots in India is more than 1500 tones and the annual requirement is about 7000 tones necessitating the increase in its cultivation and higher production.

1.2.2. Morphology

A dense, hairy erect grayish to mentose herb or under shrub. The roots are stout, long tuberous, fleshy, whitish brown and aromatic. The leaves are simple, alternate or sub-opposite, round-oval shaped. The flowers are greenish-yellow and found in few flowered clusters in axils. The fruit is a round orange-red berry, enclosed in green enlarged calyx. The fruit resembles that of red cherries. The seeds are many, yellow kidney shaped and discoid.

1.2.3. Taxonomical Classification

- Kingdom : Plantae (Plants)
- Sub kingdom : Tracheaobionta (Vascular Plant)
- Super division : Spermatophyta (Seed Plants)
- Division : Magnoliophyta (Flowering Plants)
- Class : Magnoliopsida (Dicotyledone)
- Sub class : Asteridae
- Order : Solanales
- Family : Solanaceae
- Genus : *Withania*
- Species : *Withania somnifera*

1.2.4. Chemical Constituents

The methanol, hexane and diethyl ether extracts from both leaves and roots of Ashwagandha were found. Alkaloid percentage in roots ranges from 0.13 to 0.31%. The roots of *Withania somnifera* are alterative, aphrodisiac, deobstruent, diuretic, narcotic, sedative and restorative in nature. The pharmacological activity of the root is attributed to the alkaloids and steroidals lactones.
. Indian ginseng's pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D.

1.2.5. Ashwagandha As Medicinal Herb

Ashwagandha is considered to be one of the best rejuvenating agents in Ayurveda. Its roots, seeds and leaves are used in Ayurvedic and Unani medicines. Ashwagandha root drug finds an important place in treatment of rheumatic pain, inflammation of joints, nervous disorders and epilepsy. Dried roots are used as tonic for hiccup, cold, cough, female disorders, as a sedative, in care of senile debility, ulcers, etc. Leaves are applied for carbuncles, inflammation and swellings. Leaf juice is useful in conjunctivitis.
Ashwagandha has anti-inflammatory, anti-tumor, anti-stress, antioxidant, mind-boosting, immune-enhancing, and rejuvenating properties. Ashwagandha root has also been noted to have sex-enhancing properties.

1.2.6. Medicinal Properties

- It has anti-stress, adaptogenic, aphrodisiac, sedative, diuretic, antispasmodic, germicidal, anti-inflammatory action.
- It is a nervine tonic.
- It enhances immunity and endurance.
- It is a natural nutrient for insomnia
- It is good hypnotic in Alcoholism.
- It is bitter in taste and hot in potency so it alleviates vata and kapha.
- It stimulates thyroid activity.
- Enhances anti-peroxidation of liver.

CHAPTER - II

2. Materials & Methods

2.1 Materials Required

- **Microorganisms:**
  E.coli Culture, *Staphylococcus aureus*, MTCC (Microbial type culture collection)
- **Animals Used:**
  Swiss Albino Mice (25 gm)
- **Glasswares:**
  Petri plates, Pipettes (1ml & 2ml), Measuring cylinder, Flask, Beaker, Jam bottles, Glass rod, Volumetric Flask, Test tubes, Conical Flask, Funnel
- **Miscellaneous:**
  Cotton, Inoculation loop, Whattmann filter paper, Centrifuge tubes, Micropipettes, Disk, Tips, Forceps, Hi Media antibiotic Zone Scale (for Zone measurement), Dropper, Aluminum foil, Rubber band, Glossy papers, Pipette bulbs, test tube stand, Wash Water, Glass slide, Ice pack.
- **Chemicals Required:**
  95% Ethanol, Distilled water, Nutrient Broth (NB), Agar, Nutrient Agar Media (NAM), Culture, Herbal Drug powder (Ashwagandha), Chloroform, Methanol, Petroleum ether, Fehling solution A & B, Ferric chloride, Mayer's reagent (Mercuric Chloride, Potassium Iodide), Ninhydrin solution, DPPH **(**Diphenyl picryl Hydrazine), Sodium Hydroxide, Biuret Reagent, Conc. Sulphuric Acid, Acetic Acid, Dilute Hydrochloric Acid, Diclofenac Sodium.
- **Instruments:**

SL. NO.	NAME OF INSTRUMENTS	PURPOSE
01.	Soxhlet Assembly (J-Sil, 50/42, Borosil glass)	For extracting the phytochemicals of powdered drug with the help of solvents.
02.	Vacuum Rotary Evaporator (Scientech)	For evaporating the phytochemicals present in the extraction.
03.	Digital Balance (Denver, Germany)	For weighing chemicals in micro quantities.
04.	Hot Air Oven (Scientech, 325 L)	For sterilizing the glass wares after washing.
05.	Laminar Air Flow Chamber Horizontal	For maintenance of aseptic condition
06.	Incubator (Scientech)	For the growth of the microorganism.
07.	Cyclo Mixer (REMI)	For mixing the suspensions
08.	Antibiotic Zone Scale Laboratories Ltd	For the measurement of zone of inhibition.
09.	Eddy's Hot Plate (Analgesitometer)	For the measurement of paw licking & jumping effect in mice.

2.2. Methodology

2.2.1. Sample Collection

The sample which was collected from Vindhya Herbals at Sanjeevani Ayurveda, Bhopal is as follows:

SL. No.	Botanical Name	General Name	Net Content
01.	*Withania somnifera*	Ashwagandha	200 gm.

Then the sample was weighed accurately in digital balance for further extraction process.

2.2.2 Extraction Methods Used

2.2.2.1. Soxhalation

250 ml of solvent (Chloroform, Methanol, Petroleum ether) was taken in a round bottom flask. Then 25gm of the drug powder was weighed in a digital weighing machine and wrapped in a filter paper to make a thimble. It was then placed in the central compartment & it was heated at a temperature range between 50^{o}-60^{0}C in a heating mantle. After heating the vapour passes through the side arm up into the reflux condenser. Here the vapour condenses, liquefies & drips into the thimble containing the material to be extracted. The warm solvent percolates through the material & the wall of the thimble & the extract gradually collects in the central compartment. Once the height of the extract reaches the top of the siphon, the entire liquid in the central compartment flows through this & back into the lower round bottomed flask. Then the process is further repeated as required.

In this method the extract gets collected in the lower vessel and gradually becomes more & more concentrated. When the drug powder was completely extracted, the solvent collected in the middle compartment displayed transparent colour. Assuming that there are no volatile substances present, the vapourisation from the heated extract is pure solvent in the vapour form & so the liquid dripped into the material from the condenser is essentially pure solvent, though derived from the extract, thus although a relatively small volume of solvent is needed. The effective volume of solvent used for the extraction is proportional to the time for which the process is allowed to continue. The extraction process was repeated for Chloroform, Methanol and Petroleum ether.

2.2.2.2. Recovery of Solvent by Rotary Vaccum Evaporator

A Rotary vacuum evaporator consists of a water bath that is heated up to vaporize the solvent. The extract was then taken in a round bottomed flask under vacuum & the vapours were trapped by a condenser and were collected for reuse. The overall process takes place in vacuum which helps to prevent oxidation.

The extracted residue was further mixed with chloroform water (0.25 ml of Chloroform in 100 ml of water) & resulted extract were stored in a refrigerator for further studies.

2.2.3. Phytochemaical Examination of Drug

Phytochemical examinations were carried out for all the extracts as per the standard Methods (Brain & Turner 1975, Evans 1996).

- Plant Constituents ----- Alkaloids
  Test / Reagent Used ----- Mayers Reagent
- Plant Constituents ----- Carbohydrates & Glycosides
  Test / Reagent Used ----- Fehling Solution
- Plant Constituents ----- Phenolic Compounds & Tannins
  Test / Reagent Used ----- Ferric Chloride Solution
- Plant Constituents ----- Flavonoids
  Test / Reagent Used ----- Alkaline Reagent Test
- Plant Constituents ----- Phytosterols
  Test / Reagent Used ----- Liebermann Burchard's Test
- Plant Constituents ----- Terpenoids
  Reagent / Test Used ----- Acidic Reagent Test
- Plant Constituents ----- Saponins
  Reagent / Test Used ----- Foam / Froth Test

2.2.4. Anti-Bacterial Activity by Disc Diffusion Method

- **Preparation of Inoculum**
  E.coli and *S.aureus* strains were used. 50ml of Nutrient broth was prepared in 100ml conical flask. It was sterilized & then inoculated with inoculum with the help of sterile loop in laminar air flow from preserved slants. They were then kept in incubator at 37^{o}c for sufficient period of time for organism to grow.
- **Preparation of Media**
  200ml of NAM (Nutrient Agar Media) was prepared and the pH was maintained at 7 to 7.2.
- **Pour Plate Method**
  1ml of prepared inoculum was poured in sterile Petri dish & then 15ml of NAM was poured in it & allowed to solidify.

- **Disc Diffusion Method**
  After solidification the disc of whatmann filter paper imbibed with 20 µl plant extracts were carefully placed with the help of forceps at the centre of the Petri dish and then kept in incubator for 24hrs.
- **Measurement of Zones**
  With the help of antibiotic zone scale the zone of inhibition (ZOI) were measured.

2.2.5. Anti-Oxidant Activity Using DPPH (Diphenyl Aicryl Hydrazine) Method

2.2.5.1. Preparation of Reagent

DPPH Reagent ----- 4 mg of DPPH was taken & dissolves in 100 ml of Methanol.
Ascorbic Acid ----- 0.1gm of Ascic acid iorbn 100 ml of distilled water.

2.2.5.2. Method

11 clean test tubes were taken and ascorbic acid solution was added to each of the test tubes in an increasing amount from 0.2, 0.4,….. The eleventh test tube was kept blank with no ascorbic acid. Then methanol was added to make the final volume to 2 ml. Then 0.5 ml of DPPH solution was added to each of the test tubes. The test tubes were allowed to stand for the reaction to occur for 10 min in dark conditions. Finally the readings were noted down by the help of UV VIS SHIMADZU 1800 Spectrophotometer at 517nm. In case of extracts obtained from herbal sample same procedure was used .20 µl of the samples were taken & volume was made to 2 ml with methanol. 0.5 ml of DPPH solution was added to each of the test tubes and it was allowed to stand for reaction for 10 min in dark conditions. Reading was noted down on UV VIS SHIMADZU 1800 Spectrophotometer at 517nm.
Determination of percentage inhibition of DPPH Activity by using following formula :
% Inhibition of DPPH Activity = A-B/A *100
Where,
A = Optical Density (O.D.) of the blank
B = Optical Density (O.D.) of the sample

2.2.6. Biological Activity

2.2.6.1. Analgesic Activity of *Withania Somnifera*

- **Animal Care and Handling:**
  The experiment was carried out on Swiss albino mice of 4 months, of both sexes, weighing between 25 to 30 gm. They were provided from Truba Institute of Pharmacy, Bhopal, (M.P.). The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44 –56% and light and dark cycles of 12:12 hours, fed with standard pallet diet and water *ad libitum* during experiment. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (Approval no. 1196/a/08/CPCSEA).
- **Chemicals:**
  Diclofenac sod. injection was purchased from Rajshree medical store, Bhopal. All other chemicals used for this study were of analytical grade.
- **Hot Plate Method:**
  The hot plate consists of an electrically heated surface. The temperature was controlled for 50° to 55 °C. The mice were placed individually on the hot plate and the time until either licking or jumping occurs was recorded by a stop-watch. (Kulkarni s.k, *2007,* Vogel, 2002*)*

2.2.6.1.1. Experimental Design

In the experiment, a total of 12 mice were used. The mice were divided into 6 groups comprising of 2 animals in each group as follows:

- **Group I:** Control
- **Group II:**
  Mice received Diclofenec sod. (10mg/kg, p.o.) only 1 day, before 1hr of placing in hot plate.
- **Group III:**
  Mice received Aqueous Extract of *W. somnifera,* (100mg/kg p.o.) once daily for 2 days.
- **Group IV:**
  Mice received Methanolic Extract of *W. somnifera,* (100mg/kg p.o.) once daily for 2 days.
- **Group V:**
  Mice received Chloroform Extract of *W. somnifera,* (100mg/kg p.o.) once daily for 2 days.
- **Group VI:**
  Mice received Pet. Ether Extract of *W. somnifera,* (100mg/kg p.o.) once daily for 2 days.

2.2.6.2. Anti-Inflammatory Activity

- **Animal Care and Handling:**
  The experiment was carried out on Swiss albino mice of 4 months, of both sexes, weighing between 25 to 30 gm. They were provided from Truba Institute of Pharmacy, Bhopal, (M.P.). The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44 –56% and light and dark cycles of 12:12 hours, fed with standard pallet diet and water *ad libitum* during experiment. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. 1196/a/08/CPCSEA)
- **Chemicals:**
  Carrageenan was purchased from Himedia. Diclofenac sodium injection was purchased from Rajshree medical store, Bhopal. All other chemicals used for this study were of analytical grade.
- **Carrageenan-Induced Paw Edema:**
  Acute inflammation was caused by injecting 0.1 ml of 1 % (w/v) carrageenan in saline into the sub-plantar region of the right hind paw of each mice. The paw volume was measured plethysmometrically at 1 h, 2h and 3 h after the carrageenan injection. Edema was expressed as mean increase in paw volume relative to control animals.

2.2.6.2.1. Experimental Design

In this experiment, a total of 10 mice were used. The mice were divided into 5 groups comprising of 2 animals in each group as follows:

- **Group I:** Control, 0.1 ml carrageenan injected in right hind paw
- **Group II:** Standard, mice received Diclofenac sodium (20mg/kg, i.p.) + 0.1 ml carrageenan injected in right hind paw.
- **Group III:** Mice received methanolic Extract of Ashwagandha, (100mg/kg p.o.) once daily for 5 days + 0.1 ml carrageenan injected in right hind paw.
- **Group IV:** Mice received chloroform Extract of Ashwagandha, (100mg/kg p.o.) once daily for 5 days + 0.1 ml carrageenan injected in right hind paw.
- **Group V:** Mice received pet. ether Extract of Ashwagandha, (100mg/kg p.o.) once daily for 5 days + 0.1 ml carrageenan injected in right hind paw.

CHAPTER – III

3. Results & Discussion

3.1. The samples were analysed for the pharmacognostic activity.

PHAMACOGNOSTIC CHARACTERISTICS OF THE SAMPLES

Scientific Name	Common Name	Part of the Plant Used	Colour	Odour	Taste	Texture
Withania somnifera	Ashwagandha	Roots	Light Cream	Sweet Aromatic	Neutral	Granular

COLOUR OF SUCCESSIVE EXTRACTS

SL. No.	Name of Reagent	Name of Drug	Colour of Extract
01.	Chloroform	*Withania somnifera*	Pale Green
02.	Petroleum Ether	*Withania somnifera*	Colourless
03.	Methanol	*Withania somnifera*	Brown
04.	Aqueous	*Withania somnifera*	Light Yellow

3.2. The extracts were then analysed for the phytochemical constituents.

PHYTOCHEMICAL ANALYSIS OF DRUG EXTRACT

Phytochemical Tests	**Ashwagandha (*Withania somnifera*)**			
	Chloroform	**Methanol**	**Petroleum Ether**	**Aqueous**
Alkaloids (Mayers Reagent)	+	+	+	-
Carbohydrates & Glycosides (Fehling Solution)	+	-	-	+
Phenolic compounds & Tannins (Ferric Chloride Test)	+	+	+	-
Flavonoids	+	+	+	-
Steroid	+	+	-	+
Terpenoids	+	+	+	-
Saponins	-	+	-	+

Table 1: Phytochemical Analysis Test Chart of Withania somnifera
(+) --- Positive
(-) ---- Negative

3.3. The Anti-Bacterial Activity of the Extracts Were Then Measured By Disc Diffusion Method.

3.3.1. Anti-Bacterial Activity of Drug Extract From Soxhlate Extraction Method

Chloroform Extract

SL. No.	**Name of the Drug**	**Micro-organism**	**Zone of Inhibition (in mm)**
01.	*Withania Somnifera*	*E. coli*	08 mm
		S. aureus	14 mm

Table 2: Anti-Bacterial Activity of Chloroform Extract of Withania somnifera

Petroleum Ether Extract

SL. No.	**Name of the Drug**	**Micro-organism**	**Zone of Inhibition (in mm)**
01.	*Withania Somnifera*	*E. coli*	06 mm
		S. aureus	No ZOI

Table 3: Anti-Bacterial Activity of Petroleum Ether Extract of Withania somnifera.

Methanol Extract

SL. No.	Name of the Drug	Micro-organism	Zone of Inhibition (in mm)
01.	*Withania Somnifera*	*E. coli*	17.66 mm
		S. aureus	15.33 mm

Table 4: Anti-Bacterial Activity of Methanol Extract of Withania somnifera.

Aqueous Extract

SL. No.	Name of the Drug	Micro-organism	Zone of Inhibition (in mm)
01.	*Withania Somnifera*	*E. coli*	10 mm
		S. aureus	No ZOI

Table 5: Anti-Bacterial Activity of Aqueous Extract of Withania somnifera.
ZOI - (Zone of Inhibition)

3.3.2. Anti-Bacterial Activity of Some Standard Antibiotics

SL. No.	Micro-organism	Zone of Inhibition (mm)	
		P10	OFX5
01.	*E. coli*	18 mm	19 mm
02.	*S. aureus*	17 mm	20 mm

- P10 - Penicillin G
- OFX5 - Oflaxacin

3.3.3. Discussion and Conclusion

Though the plant powder was procured from the authentic source but still for the confirmation we have done the organoleptic study under Pharmacognostic characterization of Drug.

The powdered drug was subjected to successive extraction protocol soxhalation. The extract so obtained was tested for the presence of phytochemical like alkaloid, carbohydrate, amino acid, Glycosides, Phenolic compounds and Tannins.

The anti-bacterial activity of the powder extract was done with Chloroform, methanol and petroleum ether. The results indicate that the antimicrobial activity of the methanolic extract of Ashwagandha was comparable with standard antibiotic. This shows the Ashwagandha has an anti-bacterial activity and this may be due to the extracted phytochemicals in methanolic extract. According to Mirjalili et.al (2009) the important compounds withferin and withanolides were isolated from the methanolic extract of the roots of the *Withania somnifera*. But further chemical characterization is needed to confirm the molecule responsible for the activity.

The anti-bacterial activity of this herbal formulation was comparable with standard antibiotics like Penicillin G and Oflaxacin.

3.4. Anti-Oxidant Activity of Withania Somnifera

Phytochemical screening reveals that the major constituents of Ashwagandha extract are phenolic compound, glycosides, alkaloid and flavanoid. Among these phenolic compounds which may be responsible for the activities of antioxidant.

3.4.1. DPPH Radical Scavenging Activity

Ashwagandha had significant scavenging effect on the DPPH free radical which increased with increasing concentration. The scavenging effect of sample was lower than that of Ascorbic acid.

SL. No.	Volume of Sample (200µl)	Volume of Methanol (in ml)	Volume of DPPH (in ml)	Absorbance (at 517 nm)	Percentage (%) of Inhibition
01.	Petroleum Ether	2 ml	0.5	0.214	49.4
02.	Chloroform	2ml	0.5	0.400	54.8
03.	Methanol	2 ml	0.5	2.215	97.2

Table 6: Observation Table of DPPH Method for Determining the Percentage of Inhibition

3.4.2. Discussion and Conclusion

The results of this study clearly indicate that Ashwagandha have high antioxidant activity and radical scavenging activity against various antioxidant systems in vitro. These assays have important applications for the food and pharmaceutical industry. Moreover, Ashwagandha can be used as an easily accessible source of natural antioxidants and as a possible food supplement.

In our present study we conclude that Ashwagandha has good antioxidant property and could be attributed to the presence of flavonoids, alkaloids, tannins, saponin glycosides and phenolic compounds. It was already reported that naturally occurring phenolic compounds have free radical scavenging property.

3.5. Analgesic Effect of Different Extracts of Withania Somnifera in Mice

GROUPS	ANIMALS	REACTION TIME (Sec.)			
		Paw Licking	Mean	Jump Response	Mean
Group I (Control)	01	56	71	68	83
	02	86		98	
Group II (Diclofenac sodium)	01	110	107	117	116
	02	104		115	
Group III (AEWS) 100mg/kg	01	59	49.5	67	58.5
	02	40		50	
Group IV (MEWS) 100mg/kg	01	71	64.5	79	71.5
	02	58		64	
Group V (CEWS) 100mg/kg	01	93	90	100	97
	02	87		94	
Group VI (PeEWS) 100mg/kg	01	90	82	98	91
	02	74		84	

Table 7: Analgesic Effect of Different Extracts of Withania somnifera Using Hot Plate In Mice

- **AEWS** - Aqueous extract of *Withania somnifera.*
- **MEWS** - Methanolic extract of *Withania somnifera.*
- **CEWS** - Chloroform extract of *Withania somnifera.*
- **PeEWS** - Petroleum Ether extract of *Withania somnifera.*

3.5.1. Discussion and Conclusion

Pain and inflammation are associated with pathology of various clinical conditions like arthritis, cancer, and vascular diseases (Weitzmann, S.A, 1990). In various traditional medicinal systems a number of natural products are used to relieve the symptoms of pain. The hot plate method has been found to be suitable for evaluation of analgesics (Woolfe, G, 1969). The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances such as PGs may be minimized by this model.

The *Withania somnifera* exhibited analgesic activity of different extract in animal model of pain (Table - 7).

Experimental studies revels that extracts of *Withania somnifera* (at dose 100 mg/kg) produced an analgesic action by increasing the time of paw licking and jump response in the model of pain in mice. Both chloroform and petroleum ether extract of *Withania somnifera* (at dose 100mg/kg) exhibits strong analgesic activity as compared to aqueous and methanolic extract (100mg/kg). Standard drug diclofenac sodium showed maximum analgesic action. Further studies are needed to isolate the active principles of the plant extract.

3.6. Anti-Inflammatory Activity of Withania Somnifera

The effect of different extracts of Ashwagandha on Carrageenan Induced Paw edema in mice.

GROUPS	ANIMALS	PAW VOLUME (mm)		
		1h	2h	3h
Group I (Normal Control)	01	01	02	02
	02	01	02	03
Group II (Diclofenac sodium)	01	02	01	01
	02	02	01	01
Group III (MEWS) 100mg/kg	01	02	02	03
	02	01	02	02
Group IV (CEWS) 100mg/kg	01	01	03	01
	02	02	01	01
Group V (PeEWS) 100mg/kg	01	02	01	01
	02	02	01	01

Table 8: Effect of Different Extract of Withania somnifera on Carrageenan Induced Paw Edema in Mice

- MEWS - Methanolic extract of *Withania somnifera.*
- CEWS - Chloroform extract of *Withania somnifera.*
- PeEWS - Petroleum Ether extract of *Withania somnifera.*
- AEWS - Aqueous extract of *Withania somnifera.*

3.6.1. Discussion and Conclusion

Carrageenan-induced paw edema is the standard experimental model for acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover the experimental model exhibits high degree of reproducibility. The development of edema has been described as biphasic (Vinegar R,1969). The first phase (1h) is mediated through the release of serotonin and histamine and the second phase (over 1h) is mediated by prostaglandins, cyclooxygenase products. Continuity between the two phases is provided by kinins (Periyanayagam JB, 2006)

The effect of different extracts of Ashwagandha is shown in Table - 8.

Experimental studies revels that extracts of *Sphaeranthus indicus* Ashwagandha (at dose 100 mg/kg) produced an anti-inflammatory action by decreasing the paw volume in the model of carrageenan-induced paw edema in mice. Both chloroform and petroleum ether extracts of Ashwagandha (at dose 100mg/kg) exhibits strong anti-inflammatory activity as compared to methanolic extracts (100mg/kg). Standard drug diclofenac sodium showed maximum anti-inflammatory action. Further studies are needed to isolate the active principles of the plant extract.

CHAPTER – IV

4. Future Prospects

The Herbal formulations have its own importance and advantages as compare to any other forms of medicines. As discussed in the present research the herbal formulations are free from any undesirable side effects and more or less they are non habit forming.

The Indian climate favours the growth of many rare varieties of medicinal Plants. But the need of the hour is, these plants should be identified and much extensive research should be done on it so that new Drug discovery can be made to cure many threatful diseases.

Many research organizations and Industries are pursuing research on exploring the flora like CIMAP, Himalayan Drugs etc. and many success stories are daily published. But the research should be carried out in a large scale and should be region specific so that new formulations can be prepared. Much work is also going on Polyherbal Formulation, in which many herbal drugs are scientifically mixed to get the synergistic effect.

The present study was focused on exploring *Withania somnifera* its different potentials. It has been observed that *Withania somnifera* shows the anti-bacterial activity. The present study will be helpful in preparing new Polyherbal formulation for anti-bacterial activity by incorporating the components of *Withania somnifera*. But much research on other activity of *Withania somnifera* can be done to generate a complete profile. The present study was also focused on anti-inflammatory and anti-oxidant potential of *Withania somnifera.*

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