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The Evaluation of Ethanolic Extract of Plant Piper Attenuatum for Asthma and Muscle Spasm

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Abstract
The study evaluated the anti-asthmatic and muscle relaxant potential of Piper attenuatum (B.Ham.) on an animal model to justify its traditional use. Despite the introduction of synthetic drugs, none have proven full efficacy and safety. The ethanolic extract of Piper attenuatum was found to contain carbohydrates, glycosides, alkaloids, steroids, saponins, flavonoids, and proteins. It significantly inhibited histamine-induced contraction in isolated Guinea pig ileum preparation, indicating its H1 receptor antagonist property. The extract of Piper attenuatum 50, 100 & 200 mg/kg p.o. exhibited significant prolonged the latent period of convulsion followed by exposure to histamine aerosol. The maximum % protection is 88.36 of standard drug followed by 71.43, 66.96, 55.65 of P.A 50, 100, 200 mg/kg dose. Skeletal muscle relaxant activity In the case of rota rod, it was observed that the 100 mg/kg body weight showed good skeletal muscle relaxant activity when compared to control.

Keywords: Evaluation, Ethanolic Extract, Piper Attenuatum, Asthma, Chlorpheniramine Histamine, Diazepam

1. Introduction

The demand for Ayurvedic products in Western countries has surged due to the side effects of allopathic drugs, leading to a focus on manufacturing Ayurvedic products by pharmaceutical companies. Ayurvedic treatment is commonly used in India, promoting local knowledge and taxonomic literature. Ayurveda, a medical system in India dating back four thousand years, uses plant alkaloids as active ingredients in its drugs. Today, the pharmacological properties of these medicines are being determined, with nearly 3,000 species used in the medicinal field.

Detailed research on the chemistry and pharmacology of products of plant origin are much essential and this may eventually lead to the discovery of medicine that can be used in the treatment of several diseases. Moreover, these local Ayurvedic preparations are scientifically evaluated and disseminated properly, our indigenous population can be given better access to efficacious drug treatment and improved health status. However, over commercial exploitation of these plant (herbal) products frequently degradation of natural resources are reported to be major threats to medicinal plants in India.[1]

There are many traditional systems of medicine in the world, each with different associated philosophies and cultural origins. Some of these, such as Tibetan traditional medicine, remain relatively localized in their country of origin while others such as Ayurvedic and Chinese traditional medicines are increasingly used in

many different areas of the world Ayurveda is the most widely practiced of the Indian traditional medicine systems, but there are others such as Siddha and Unani which are also used in the Indian subcontinent.
Herbal care or traditional system of medicine are used throughout the world and from centuries herbs have been the original source for most of the drugs. Medicinal plants contain so many chemical compounds which are the major source of therapeutic agents to cure human diseases. Recent discovery and advancement in medicinal and aromatic plants have lead to the enhancement of health care of mankind.[2]
Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (UNESCO, 1996). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998).
Moreover, in these societies herbal remedies have become more popular in the treatment of minor ailments & also on account of the increasing costs of personal health maintenance. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity.
About 1400 herbal preparations are used widely, according to a recent survey in Member States of the European Union. Herbal preparations are popular and are of significance in primary healthcare in Belgium, France, Germany and the Netherlands. Such popularity of healthcare plant-derived products has been traced to their increasing acceptance and use in the cosmetic industry as well as to increasing public costs in the daily maintenance of personal health and well-being. Examples of such beauty-oriented therapeutically are skin tissue regenerators, anti-wrinkling agents and anti-age creams. Most dermaceuticals are derived from algal extracts that are rich in minerals and the vitamin B group. Skincare products such as skin creams, skin tonics, etc. derived from medicinal plants are grouped together as dermaceuticals. Also, amongst the poor, cures and drugs, derived from plants, constitute the main source of healthcare products.
As defined by WHO “Health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity”. Market and public demand for herbal medicines has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity[3].
Use of herbs and traditional systems of medicine is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show their value in the treatment and prevention of diseases[4].
Herbal drugs constitute a major share of all the officially recognized systems of medicine in India they are Ayurveda, Yoga, Siddha, Homeopathy and naturopathy.
1.1 billion Populations still use these non-allopathic systems of medicine. Currently there is no separate category of herbal drugs or dietary supplements, as per the Indian Drug Act. However, there is a vast experimental evidence base for many of the natural drugs. This offers immense opportunities for Observational Therapeutics and Reverse Pharmacology. Clinical research has been carried out on the medicinal plants and their formulation[5].
Many of the herbs and spices used by humans to season food yield useful medicinal compounds. Similarly to prescription drugs, a number of herbs are thought to be likely to cause adverse effects. Furthermore, "adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal[6].
The valuable medicinal properties of different plants are due to the presence of several constituents i.e. saponins, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, Sesquiterpenes lactones, terpenoids and phorbol esters[7].

1.1 Asthma

Asthma is an inflammatory disease caused by dysregulated immune responses in the airway mucosa, with characteristic features including airway inflammation; excessive airway mucus production due to goblet cell hyperplasia and thickening of the airway wall.[8] Airway inflammation is associated with the infiltration of

eosinophils, neutrophils, and T and B lymphocytes into airway and lung tissues. CD4+ T cells producing Th2 cytokines play an important role in the lungs of asthmatic subjects, particularly because interleukin-4 (IL-4) and interleukin-13 (IL-13) enhance immunoglobulin E production, interleukin-5 (IL-5) enhances eosinophil accumulation, and IL-13 directly enhances mucus Hyper secretion and airway hyperresponsiveness.[9, 10] IL-5 is a central factor mediating eosinophil expansion, recruitment and prolonged tissue survival in response to allergic stimuli. It seems reasonable to assume that blockade of IL-5 would result in elimination of eosinophilic associated with allergic asthma. Cytokine receptor antagonists to IL-4, IL-5 and IL-13 have been tested for their ability to control the balance between Th1 and Th2 responses. However, these reagents are not orally active and often cause undesirable side-effects. Thus, there is a need for development of an orally active and safe immune modulator for the treatment of allergic asthma. We therefore screened for natural reagents from plants based on Korean traditional herb medicines. Piperine, a main component of Piper longum Linn. is a plant alkaloid known to exhibit a variety of biological activities. Piperine was the first amide to be isolated from piper species and was reported to display anti-inflammatory activity.[11]

Other biological activities of Piperine include immunomodulatory properties,[12] a consistent immunosuppressive effect[13] and immunomodulatory and antitumor effects.[14] However, there are no reports of the anti-asthmatic and muscle relaxant activities of Piperine in vivo. The aim of this study was to evaluate the ability of Piperine to control Th1- and Th2-type cytokines, various immune cell phenotypes and other factors. Using a murine model of asthma, we studied the effect of Piperine on airway eosinophil accumulation, Th2 cytokine production, various immune cell phenotypes and histology. As mention in literature survey Piper attenuatum (B. Ham.) also useful in Treating Asthma[15] Convulsion, Plant also having muscle relaxant, property and CNS depressant action[16]

1.2 Muscle Relaxant

The earliest known use of muscle relaxant drugs dates back to the 16th century, when European explorers encountered natives of the Amazon Basin in South America using poison-tipped arrows that produced death by skeletal muscle paralysis. This poison known today as curare, let to some of the earliest scientific studies in pharmacology. It is active ingredient, Tubocurarine the alkaloid curarine is transported in bamboo tubes so it is known as tubocurarine the active form of alkaloid dextroisomer so it is called as d-tubocurarine.[17]

Skeletal muscle relaxants are drugs that reduce the muscle tone. They act either peripherally at the neuromuscular junction (neuromuscular blockers) or centrally in the cerebrospinal axis or directly on the contractile mechanism of the muscle as with dantrolene. The main clinical use of skeletal muscle relaxants (neuromuscular blockers) is as an adjuvant in surgical anesthesia, to obtain relaxation of skeletal muscles, particularly of abdominal wall and lower limbs so that operative manipulations become easier. These drugs cause several adverse effects. Today, phytopharmaceuticals are gaining more importance. Herbal drugs can be cost-effective alternatives for the costly Western drugs. It is, therefore, necessary to acquire and preserve the traditional system of medicine by proper documentation and identification of herbal remedies.[18]

These actions of Piper Attenuatum (B. Ham.) not prove scientifically, Various traditional plants are used for the treatment of above disease instead of regular medicines due to lesser side effects and higher efficacy. Plants constituents viz alkaloids, flavonoids, amides, tannins, terpenoids are responsible for curing Asthma & Spasm.

1.3 Plant Profile

Plant Piper attenuatum (B.Ham.) is a rare piper species which is found in the tropical and sub-tropical region and mainly found in southern part of India like Kerala, and Tamilnadu and Himalayas, Assam, khasi hills, & nilgiris. Plant having important like alkaloids, amides, glycosides, tannins etc. which are responsible for its therapeutic efficacy. Piper attenuatum (B.Ham.) also known as 'P.bantamense blume' or 'Oval leaved peeper plant. Various parts of the plant P. attenuatum (Piperaceae) of genus Piperlikeseeds, root, leaves, and stem are used in different indications. Roots of the Piper attenuatum (B.Ham.) show excellent diuretic activity when macerated in water.[19]plant of Piper attenuatum (B.Ham.) showed significant anti-malarial activity.[20]

2. Material and Methodology

2.1 Materials

2.1.1 Plant

The genus Piper belongs to the family **"Piperaceae"** comprising more than 700 species distributed throughout the tropical and subtropical regions of the world.[19] Plant Piper attenuatum (B.Ham.) was procured from **TROPICAL BOTANIC GARDEN & RESEARCH INSTITUTE, PALODE, TRIVANDRUM, KERALA** and authenticated. The wet plant shed dried and converted into coarse fine powder at Alwar Pharmacy College, Alwar, Rajasthan. Extraction of the plant was carried out by using Soxhlet apparatus at Alwar Pharmacy College, Alwar.

2.1.2 Drugs

2.1.2.1 Drugs for Asthma

Chlorpheniramine
Histamine

2.1.2.2 Drugs for Muscle Relaxant

Diazepam

2.1.2.3 Chemicals

Normal saline	0.9%
Distilled Water	-
Ethyl alcohol	99.9% C.Y.C., China
Anesthetic Di-ethyl Ether	95% C. D. H. Ltd. Delhi
Alcohol	(60%, 70%, 80%)

Hydrochloric acid
Barfoed's reagent
Dragendroff's reagent
Hager reagent
Mayer reagent
Wagner reagent
Fehling A and Fehling B solution

All chemicals mentioned above were of analytical grade. Reagents were prepared according to the need and some were purchased from commercial sources.

2.1.3. ANIMALS

Three activities were carried out during the dissertation they are Anti-Pyretic, Anti-Hyperlipidemic and Anti-Diabetic Activity.

2.1.3.1 Guinea Pigs

Guinea pigs that are small, typically about 8 to 16 inches long and weighing between 1.5 and 3 pounds were used for preclinical studies.

2.1.3.2. Mice

Healthy mice of either sex weighing between 70-100 g were taken for the study. All the animals were given by Alwar Pharmacy College, Alwar. The animals were acclimatized by keeping them in the animal house facility of Alwar Pharmacy College, Alwar.

They were housed individually in polypropylene (32x24x16 cm) cages containing husk as bedding material and maintained under controlled conditions of temperature (23±2^{o}c), humidity (55±5%) and 12h light and 12h dark cycles. The animals were fed with standard pellet diet and water ad libitum. Approval of the Institutional Animals Ethics Committee (IAEC) of Alwar Pharmacy College, Alwar was taken

2.2. Methodology

The objectives of the current study was as following-

1. Extraction of **Piper attenuatum (B.Ham.)** by using the suitable solvents (e.g., alcohol and petroleum ether) and its phytochemical evaluation.
2. Evaluation of Anti-Asthmatic activity of ethanolic extract of Piper attenuatum (B.Ham.) using

(a) Isolated Guinea pig Ileum Preparation
(b) Histamine induced Bronchospasm.
4. Evaluation of Muscle relaxant activity of ethanolic extract of plant
Piper attenuatum (B. Ham.)
(a) Muscle relaxant activity using Diazepam in mice

2.2.1. Extraction
Part of Plant of **Piper attenuatum (B.Ham.)** were shade dried and powdered. The total quantity of powdered material was about 270 gm. This powdered material was subjected to defat with Petroleum Ether for 72 hours in a Soxhlet apparatus. Then after 72 hours this defatted material is subjected to extraction with ethanol (99.99%) in a Soxhlet apparatus for 48 hours. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using flash evaporator.

2.2.2 Qualitative chemical test[21,22,23]
2.2.2.1 Preliminary Phytochemical investigation of extract
Qualitative chemical tests were conducted for Ethanolic extract of Piper attenuatum (B.Ham.)to identify the various phytoconstituents. The various tests conducted are given below and observations are recorded and tabulated.

A. Tests for Carbohydrate
Molisch's test (General test):
To 2-3 ml. aqueous extract, few drops of α-naphthol solution in alcohol was added, shaken and concentrated H_2SO_4 was added from the sides of the test tube. It was observed for violet ring at the junction of two liquids.

B. For Reducing Sugars
(a)Fehling's test: 1 ml. Fehling's A and 1ml. Fehling's B solutions were mixed and boiled for one min. Equal volume of test solution was added and heated in boiling water bath for 5-10 min and observed for a yellow and then brick red precipitate.
(b) Benedict's test: Equal volumes of Benedict's reagent and test solution (T.S.) were mixed in a test tube and heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.

C. Tests for Proteins
(a) Xanthoprotein test (For protein containing tyrosine or tryptophan): Mixed 3ml. T.S. with 1 ml. concentrated H_2SO_4, observed for white precipitate.
(b) Test for protein containing sulphur: Mixed 5 ml. T.S. with 2 ml. 40% NaOH and 2 drops 10% lead acetate solution. Solution was boiled, turns black or brownish due to PbS formation.

D. Tests for Steroids:
(a) Salkowski Reaction: To 2 ml. of extract, 2 ml. chloroform and 2 ml. concentrated H_2SO_4 were added. Shook well, whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.
(b)Liebermann-Burchard Reaction: Mixed 2ml. extract with chloroform. Added 1-2 ml. acetic anhydride and 2 drops concentrated H_2SO_4 from the sides of test tube, observed for first red, then blue and finally green colour.

E. Tests for Flavonoids:

(a) Lead Acetate Test: To the small quantity of ethanolic extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoid.

(b) Ferric chloride test: To test solution, added few drops of ferric chloride solution observed for intense green colour.

F. Tests for Alkaloids

(a)Mayer's test: To 2-3 ml. T.S, added few drops Mayer's reagent and observed for precipitate.

(b)Hager's test: To 2-3 ml. T.S, added Hagers reagent and observed for yellow precipitate.

G. Tests for GlycosidesGeneral test for Glycosides

Part A: To 2-3 ml. of extract dil. H_2SO_4 was added and heated on a water bath for 1-2 min. Neutralise with 10% NaOH, check with litmus paper and to resulting solution add Fehling's A & B. Intense red precipitate in this case shows glycosides was present.

Part B: To 2-3 ml. of extract, water was added and heated. According to need, NaOH was added for neutralisation and also added equal quantity of water. To the resulting solution added Fehling's A & B. Increased red precipitate in this case showed glycosides was absent.

H. Tests for Cardiac Glycosides

(a)Legal's test: (For cardenoloids) to aqueous or alcoholic test solution, added 1 ml. pyridine and 1 ml. sodium nitroprusside, observed for pink to red colour.

(b)Test for deoxysugars: (Kellar Killani test) To 2 ml. extract added glacial acetic acid, one drop of 5% $FeCl_3$ and concentrated H_2SO_4, observed for reddish brown colour at junction of the two liquids and the upper layer for bluish green color.

(c) Libermann's test: (For bufadenolids) Mixed 3 ml. extract with 3 ml. acetic anhydride. Heated and cooled. Added few drops concentrated H_2SO_4 and observed for blue colour.

I. Tests for Saponins Glycosides

(a) Foam test: The drug extract or dry powder was shaken vigorously with water and observed for persistent foam.

(b)Haemolytic test: Added test solution to one drop of blood placed on glass slide and observed for appearance of haemolytic zone.

J. Solubility

Solubility of Ethanolic extract of Piper attenuatum (B.Ham.) was checked out in water as well as normal saline.

2.2.3. Extract

Ethanolic extract of Plant was used to evaluate Anti-Asthmatic (200 mg/kg), Muscle relaxant (200 mg/kg) activities Stock solution of the extract was prepared in the range of 200-400 mg/ml. in saline according to the need of study.

2.2.4. ACTIVITIES

2.2.4.1. Anti-Asthmatic Activity

Anti-Asthmatic Activity were carried out by using these models

Evaluation of Anti Asthmatic activity using isolated Guinea pig Ileum Preparation

Guinea pigs were (Overnight fasted) were scarified and ileum was mounted in an organ bath containing tyrode solution which was continuously aerated at 37±0.5^{O}C. Bioassay of histamine 10microgram/ml in plain tyrode solution containing 100 microgram/ml. percentage maximum contractile response was plotted to generate dose response curve in the absence and presence of plant extract.

Histamine induced Bronchospasm

Guinea pigs were divide into 4 groups each contain 5 animals respectively. Control group received 5% tween 80 other groups received ethanolic extract of Piper attenuatum (100 & 200 mg) and standard group received CPM (2 mg/kg). Prior and after drug treatment each animal was placed in histamine chamber & exposed to .2 % histamine aerosol. PCT was determine from the time of exposure to onset of Dyspnea

leading to the appearance of preconvulsive dysponea in sec. the % age protection offered by drug in PCT was calculated for each dose and positive control was calculated by using this formula

Protection (%) = (1-T_1/ T_2) * 100

T_1- Mean of PCT before administration of test drug

T_2- Mean of PCT after administration of test drug

Statistical analysis

The results was expressed as mean ± S.E.M. the significant of various treatments was calculated using students paired t-test by using Prism 5.0[24].

2.2.4.2 Muscle Relaxant Activity

Rota rod is a horizontal metal rod coated with rubber, 3cm in diameter, put at a rotation of 25 rpm. The metal rod is about 50 cm above the surface to prevent the animal from jumping off the roller. The mice were placed on the revolving rod. The initial basal reading of the number of rotations covered by each animal before falling from the rota rod was recorded. The test and standard compound was administered 1hr before placing the rats on the rota rod. The animals falling from the rota rod within the test period was calculated for every test and standard drug concentrations and compared. Mice were divided into 3 groups consisting of 6 animals each.

Group I (Control): Received normal saline (1 ml/kg body weight).

Group II (Standard): Received diazepam (4mg/kg body weight).

Group III Received Ethanol extract of Piper Attenuatum (B. Ham) (200 mg/kg body weight).

3. Results

3.1.1. Phyto-chemical screening

Table4: Presence of Phytochemical constituent's in Ethanolic extract of Piper attenuatum (B.Ham)

Sr. No.	Name of The Test	Observation	Conclusion
1.	**Test for Carbohydrate**	All tests were positive	**Carbohydrates** were present in the Ethanolic extract
	Molisch's test		
	Fehling's test		
	Benedict's Test		
2.	**Test for protein's**	All tests were positive	**Proteins** were present in the Ethanolic extract
	Xanthoprotein Test		
	Liebermann's Test		
3.	**Test for Glycoside's**	All tests were positive	**Glycoside's**were present in the Ethanolic extract
	Killer- Killani		
	Legal Test		
4.	**Test for Alkaloid's**	All tests were positive	**Alkaloid's**were present in the Ethanolic extract
	Hager's Test		
	Mayer's		
5.	**Test for Steroid's**	All tests were positive	**Steroid's**were present in the Ethanolic extract
	Salkowski reaction		
	Liebermann-Burchard		
6.	**Test for Saponin's**	All tests were positive	**Saponins** were present in the Ethanolic extract
	Foam test		
	Haemolytic test		
7.	**Solubility**	Slightly Soluble	Soluble in Normal Saline
	In water	Soluble	
	In Saline		
8.	**Tests for Tannins and Phenolic Compounds**	All test were negative	**Tannins** and **Phenolic** Compounds were absent in the Ethanolic extract
	Lead acetate test		
	5% Fe Cl_3 test		
9.	**Test for Flavonoid's**	All tests were positive	**Flavonoid's**were present in in the Ethanolic extract
	Shinoda test		
	Lead acetate test		

	Alkaline solution		

Statistical analysis

The results was expressed as mean ± S.E.M. the significant of various treatments was calculated using students paired t-test by using Prism 5.0.[25]

3.1.2. Anti-Asthmatic Activity

(a) Evaluation of Anti Asthmatic activity using isolated Guinea pig Ileum Preparation

In the present study Ethanolic extract of Piper attenuatum (200 mg/kg) significantly p<0.01 inhibited the histamine induced contraction of isolated Guinea pig ileum Preparetion indication its H_1 rec antagonist property. There was decrease in % response in presence of P.A when compared to histamine (10) microgram/ml alone.

Table: 5 Effect of Piper attenuatum on isolated Guinea pig Ileum

		Response (%)					
Group	**Drug dose**	**0.1 ml**	**0.2 ml**	**0.4 ml**	**0.8 ml**	**1.6 ml**	**3.2 ml**
Control	Histamine (10 mc/ml)	22.50±.058	40±.088	85.0±.116	90±.089	97.5±.058	100±.08
Standard	Histamine + CPM	1.00±.003**	.90±.007**	.83±.009**	.83±.009**	4.7±.011**	7.25±.007**
P.A (200) mg/kg	Histamine + P.A (10+200 +mc/ml)	1.00±.007**	1±.006**	7.30±.019**	9.75±.009**	12.5±.058**	22.50±.058**

Values are in mean± S.E.M., One way Anova was followed by Dunnet t test where *p<0.05 & ** p>0.01as compared to control n=5

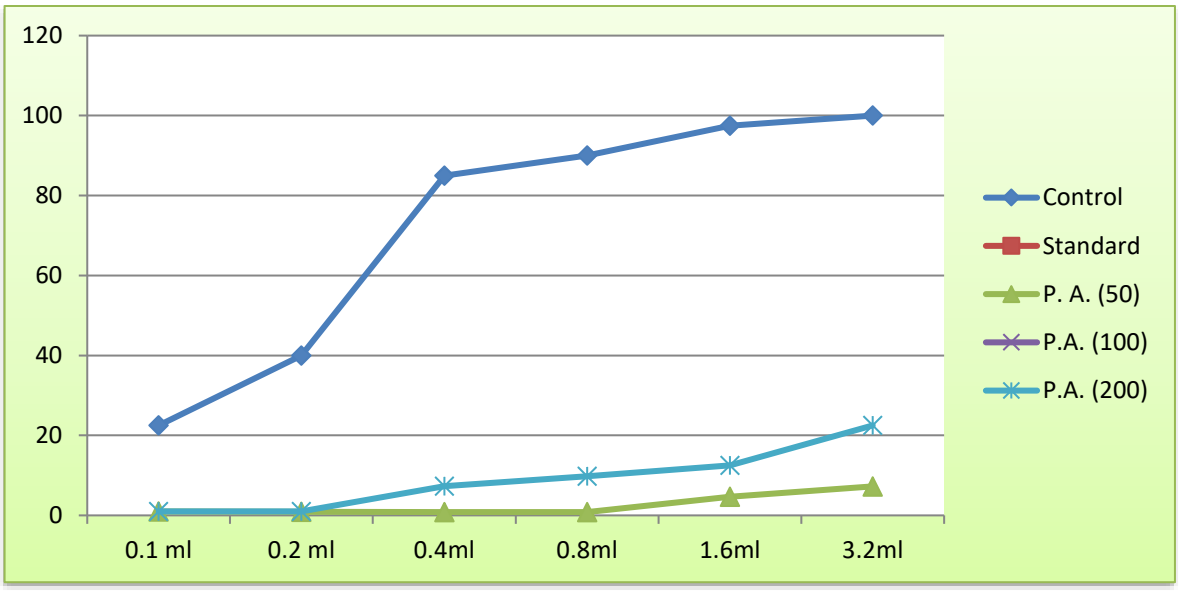

Figure: 11 Effect of Piper attenuatum on isolated Guinea pig Ileum Preparation

(b) Histamine induced Bronchospasm

Table: 6 Effect of Piper attenuatum on histamine induced bronchospasm

Values are in mean± S.E.M., One way Anova was followed by Dunnet t test where *p<0.05 & ** p>0.01as compared to control n=5

C.P.M Chlorpheniramine

P.A. Piper attenuatum

Group	Drug Dose & Route	PCT (Before)	PCT (After)	Mean Exposition time	Protection %
Control	Tween 80(10 ml/kg) p.o	85±1.09	88±1.14	3±.63	3.41
Standard	CPM (2mg/kg)	56±1.55	481±.84	425±1.19**	88.36
P.A (50)	50 mg/kg p.o.	55±1.52	124±1.09	69±1.41**	55.65
P.A (100)	100 mg/kg p.o.	74±1.67	224±1.14	150±2.35**	66.96
P.A (200)	200mg/kg p.o.	64±1.05	384±1.30	320±3.09**	71.43

The extract of Piper attenuatum 50, 100 & 200 mg/kg p.o. exhibited significant prolonged the latent period of convulsion followed by exposure to histamine aerosol. The maximum % protection is 88.36 of standard drug followed by 71.43, 66.96, 55.65 of P.A 50, 100, 200 mg/kg dose.

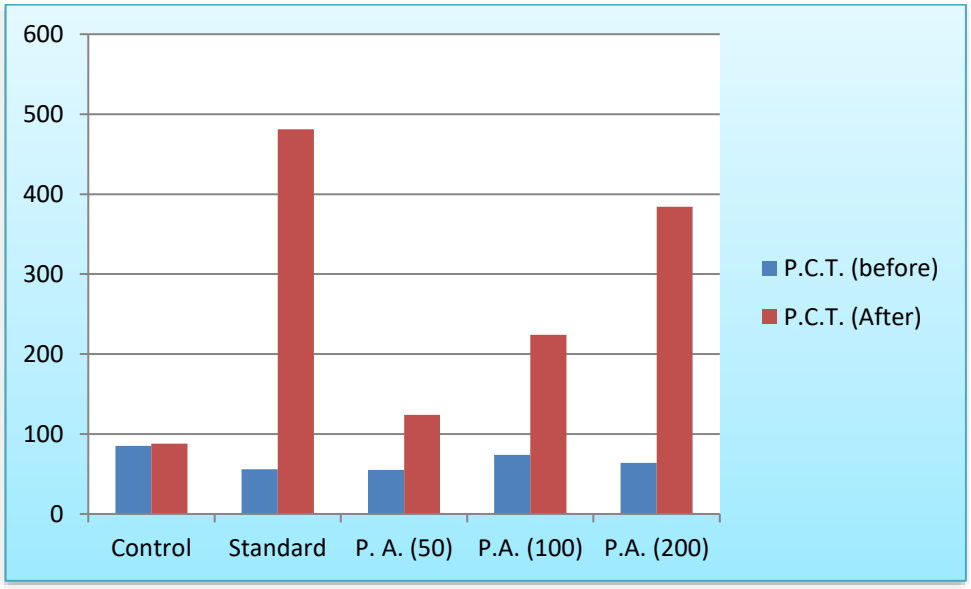

Figure: 12 Effect of Piper attenuatum on Bronchospasm

3.1.3 (a) Muscle Relaxant Activity

Table 7 Effect of Piper attenuatum (B.Ham.) extract on Muscle relaxant activity

Sr. No.	Groups	Fall off time (Sec.)		Difference	Percentage
		Before	After		
1.	Control	250.16±10	-	-	-
2.	Standard	250.16±10	25±0.853	225.16±9.147	90.006
3.	P. A. (100 mg/kg)	250.16±10	75±5.48	175.16±4.52	70.019

Data was analysed using one way ANOVA.

Skeletal muscle relaxant activity In the case of rota rod, it was observed that the 100 mg/kg body weight showed good skeletal muscle relaxant activity when compared to control.

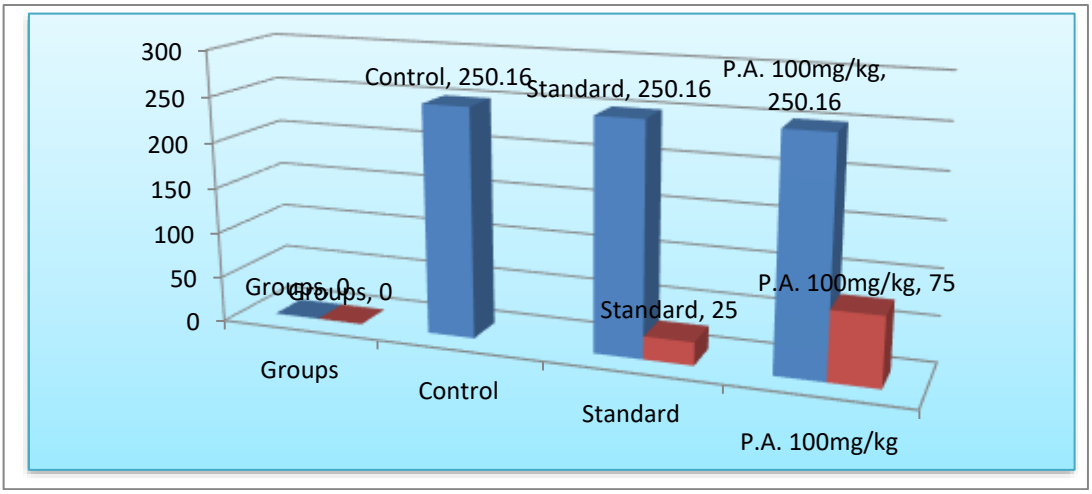

Figure: 13 Effects of Piper attenuatum on muscle relaxant activity

4. Discussion

4.1 Muscle relaxant Activity

4.1.1 Diazepam induced muscle relaxation using rotarod

A muscle cramps is defined as sudden, involuntary, spasmodic and painful contraction of skeletal muscle. It often occurs after or during sports and marathons. It is a well known clinical problem that athletes try to avoid it. The mechanism underlying muscle cramps are poorly understood. Therefore there is no evidence based way to efficiently prevent muscle cramps although it is widely known as cramping of muscles.

Piperine is an alkaloid, the piperidine amide of piperic acid. It is a major pungent principle of various Piper species such as black pepper and long pepper which are commonly used for a condiment and employed in folkloric medicine for treatment of asthma, insomnia and abdominal disorders,& muscle relaxant activity in mice;. It is also present in the leaves of Rhododendron fauriae (Ericaceae). Piperine was recently found to possess central nervous system (CNS) depressant properties.

Diazepam is the only benzodiazepine that is FDA approved for treatment of spasticity and muscle spasms. Diazepam binds to GABAA receptors and potentiates GABAergic activity by increasing chloride conductance, which results in pre-synaptic inhibition in the spinal cord. Diazepam has demonstrated efficacy in the management of spasticity associated with spinal cord injury, hemiplegia, and muscle spasm. However, it is not often recommended as a first line agent due to risks of sedation and a potential for dependence or abuse. Diazepam is metabolized to the active metabolites des methyl diazepam, temazepam, and oxazepam, the last 2 of which are available commercially. AEs include lethargy, sedation, anterograde amnesia, cognitive impairment, and dependence. Withdrawal syndrome can occur with abrupt cessation of diazepam, and may lead to seizures.

4.2 ANTI ASTHMATIC ACTIVITY

Bronchial asthma is a chronic inflammatory disease which is characterized by both Broncho constriction and airway inflammation which leads to bronchial hyper responsiveness to various stimuli in which many cell play a role more important mast cell, eosinophils and T cells. Different agonist like Ach, histamine 5-HT, Bradykinin is responsible for contractile response.

Guinea pig ileum is used for screening of anti histaminic activity. The stimulation of H_1 produced graded dose related contraction of isolated guinea pig ileum. In the present study ethanolic extract of Piper attenuatum significantly inhibit histamine induced contraction of isolated guinea pig ileum Preparation indicating its H1 rec antagonistic activity and support the anti asthmatic property of plant. There was decrease in % response in ethanolic extract of Piper attenuatum at dose of 200 mg/ml when compared to histamine alone.

5. Conclusion

Ethanolic extract of authenticated plant Piper attenuatum (B.Ham.) was obtained by **Soxhlet extraction.** Ethanolic extract of Piper attenuatum (B.Ham.) was found to contain carbohydrates, glycosides, alkaloids, steroids, saponins, flavonoids & proteins. Two activities were carried out on Ethanolic extract of Piper

attenuatum (B.Ham.) by using different models for Muscle Spasm and asthma.. Ethanolic extract of the plant show's significant results in both the activities.
It may be possible that (Piperine) and (Piperidine) are responsible for muscle relaxant and Anti Asthmatic activity.

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Conflict of Interest
The authors declare that they have no conflict of interest.

6. Reference

1. Samy RP, Pushparaj PN, Gopalakrishnakone P. A compilation of bioactive compounds from Ayurveda. Biomedical informatics 2008; 3(3): 100-110.
2. Umashanker M, Srivastava S. Traditional Indian herbal medicine used as antipyretic, antiulcer, anti-diabetic and anticancer. International Journal 0f Research in Pharmacy and Chemistry 2011; 1(4): 1152-59.
3. Singh, Scholar Satbir. (2017). GLOBAL REGULATORY CHALLENGES OF COMMON TECHNICAL DOCUMENT. World Journal of Pharmacy and Pharmaceutical Sciences. 6. 1298-1309. DOI: http://dx.doi.org/10.20959/wjpps201712-10616
4. Khaki A, Fathiazad F. Diabetic Nephropathy - Using Herbals in Diabetic Nephropathy Prevention and Treatment. 207-32.
5. Ashok DB, Thomas PA. Current status of herbal drugs in India: An overview. J clin Biochem Nutr 2007 July; 41(1): 1-11.
6. Khaki A, Fathiazad F, Ravi B, Sanjay S. Herbals in Diabetic Nephropathy Prevention and Treatment. 201-40.
7. Raju G, Maridass M. Evaluation of antimicrobial potential of piper Species. Nature of Pharmaceutical Technology 2011; 1(1): 19-22.
8. Robinson DS et al. Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 1992; 326: 298–304
9. Umetsu DT, DeKruyff RH. Th1 and Th2 CD4+ cells in human allergic diseases. J Allergy Clin Immunol 1997; 100: 1–6.
10. Wills-Karp M et al. Interleukin-13: central mediator of allergic asthma. Science 1998; 282: 2258–2261.
11. Virinder SP et al. Phytochemistry of genus Piper. Phytochemistry 1997; 46: 597–673.
12. Pathak N, Khandelwal S. Cytoprotective and immunomodulating properties of piperine on murine splenocytes: an in vitro study. Eur J Pharmacol 2007; 576: 160–170
13. Dogra RK et al. Immunotoxicological effects of piperine in mice. Toxicology 2004; 196: 229–236
14. Sunila ES, Kuttan G. Immunomodulatory and antitumor activity of Piper longum Linn. and piperine. J Ethnopharmacol 2004; 90: 339–346
15. Kim SH, Lee YC. Piperine inhibits eosinophil infiltration and airway hyperresponsivness by suppressing T cell activity and Th2 cytokine production in the ovulbumin induced asthma model. Journal of Pharmacology and Pharmacy 2009; 61: 353-59.
16. Basu BD. Compendium of Indian medicinal plants.
17. Veena NS, Sivaji K, Benergi GV, Babu MF, Kumari R. Skeletal muscle relaxant property of diazepam by using rotarod on albino mice. Indian Journal of Basic and Applied Medical Research. 2015; 4(4): 714-721.
18. Nair PV, Nair B. Muscle relaxant activity of hydroalcoholic extract of Mimosa pudica whole plant in mice. National Journal of Physiology, Pharmacy and Pharmacology 2017; 7(4): 432-437.
19. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd Ed. Dehradun: International book distributor; 2006. 2128-37
20. Bawm S, Tiwananthagoran S, Lin KS, Hirota J, Irie T, Htun LL, Maw NN, Myaing TT. Evaluation of Myanmar medicinal plants for Anti-trypsomonal & cytotoxic activities. J Vet Med Sciences 2010; 72(4): 525-28.

21. Jaiswal A, Sutar N, Garai R, Pati MK, Kumar A. Anti- pyretic activity of Platycladus Orieantalis leaves extract. IJABPT 2011; 2(1): 175-78.
22. Mazumdar M, Sasmal D, Ghosh A, Paramaguru R. Evaluation of Anti-Hyperlipidemic and Anti- oxidant activity of Pterospermum acerifolium. Pharmacology online 2011; 3: 128-46.
23. Gupta N, Bhatia V, Agarwal M, Sharma RK. A Comparative antidiabetic and hypoglycemic activity of the crude alcoholic extract of the plant leucasaspara and seed of pithecellobium begminum in rats. IJRAP 2011; 2(1): 275-80.
24. Kaushik D. Ran R, Kaushik P et. al. Invivo and invitro anti asthmatic activity of plant Piper longum. International journal of pharmacology 2012; 8(3): 192-97
25. Shetty AK et. al. Evaluation Of Skeletal Muscle Relaxant Activity Of Ethanol Extract Of Chromoleana Odorata. IOSR Journal Of Pharmacy 2017 March ; 7(3): 92-95