ISSN: 2320-9267

Indian Journal of Pharmaceutical and Biological Research (IJPBR)

Journal homepage: www.ijpbr.in

ORIGINAL RESEARCH ARTICLE

Antioxidant and Skeletal Muscle Relaxant Activity of Leaf Extract of Plant *Piper Attenuatum* (B. HAM)

Soni Gaurav[1]*, Govindasamy Jeyabalan[2], Ahuja Anil[3]

ABSTRACT

Piper attenuatum (B. Ham) is traditional medicinal plant in India. It has been claimed in traditional Indian system of medicine that the phytochemical constituents present in *P. attenuatum* (B. Ham) have Antioxidant and skeletal muscle relaxant activity. So the present study aimed to evaluate anti–oxidant and skeletal muscle relaxant activity of ethanolic, aqueous and ethanolic leaf extract of plant *P. attenuatum* (B. Ham), respectively. Antioxidant activity was evaluated by *in vitro* as well as in vivo methods. Invitro method we used DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and H_2O_2 (Hydrogen peroxide) scavenging assay while in vivo methods antioxidant enzymes like Superoxide Dismutase (SOD) and reduced glutathione (GSH) were assayed by using animal models. Anti-oxidant activity of following concentrations 10µg/mL, 20µg/mL, 30µg/mL, 40µg/mL and 50µg/mL were measured for both extracts. Ethanolic extract of *P. attenuatum* (B. Ham) was evaluated at dose of 100mg/kg & 200 mg/kg b.w. for skeletal muscle relaxant activity by using rota rod apparatus. The high antioxidant activity was found in the ethanolic extract of *P. attenuatum* (B. Ham) compared to aqueous one. For muscle relaxation, 200 mg/kg b.w. showed a significant reduction in the time spent by the animals on the revolving rod compared to the control. From the above study it may be concluded that both extract of *P. attenuatum* (B. Ham) having Anti – oxidant and skeletal muscle relaxant activity.

Keywords: Anti – oxidant activity, DPPH, *P. attenuatum* (B. Ham), Skeletal muscle relaxant activity.

Indian J. Pharm. Biol. Res. (2021): https://doi.org/10.30750/ijpbr.9.1.2

INTRODUCTION

There are many traditional systems of medicine in the world, each with different associated philosophies, some of these like Tibetan traditional medicine, remain localised in their country; while others like Ayurvedic and Chinese traditional medicines are continuously used in many different areas of the world.[1] Medicines that derived from various plants have been the first line of defence in maintaining health and combating diseases. Chemical principles from natural sources have become much simpler and contributed to the development of new drugs from medicinal plants.[2] Spices and herbs are recognized as sources of natural antioxidants and thus play an important role in the prevention of diseases and aging. The plants which are investigated, showing enormous potential, pepper family one of them.[3] Reactive oxygen species generated in cells they modulate various physiological functions and represent an essential part of metabolism. Excessive generation of these radicals disrupts the body's antioxidant defense system that causes "oxidative stress" leading to a variety of disorders like coronary heart disease, neurodegenerative disorders, diabetes, and arthritis inflammation, lung damage and many more. Antioxidants have the property to prevent oxidative damage, so the wide use of natural antioxidants replace conventional synthetic antioxidants in food and food supplements because natural products are considered to be promising and

[1]Research Scholar, Sunrise University, Bagar Rajput, Alwar, Rajasthan, India

[2]Professor and Principal, Department of Pharmacognosy, Alwar Pharmacy College, RICCO Industrial Area, MIA, Alwar, Rajasthan, India

[3]Research Supervisor, Sunrise University, Bagar Rajput, Alwar, Rajasthan, India

Corresponding Author: Soni Gaurav, 1Research Scholar, Sunrise University, Bagar Rajput, Alwar, Rajasthan, India, E-Mail: Soni.gaurav09@gmail.com

How to cite this article: Gaurav S, Jeyabalan G., Anil A.. Antioxidant and Skeletal Muscle Relaxant Activity of Leaf Extract of Plant *Piper Attenuatum* (B. HAM). Indian J. Pharm. Biol. Res. 2021; 9(1): 8-15.

Source of support: Nil

Conflict of interest: None.

Received: 18/07/2020 **Revised:** 29/10/2020 **Accepted:** 20/11/2020 **Published:** 25/03/2021

safe.[4] Antispasmodic drugs like cyclobenzaprine are used to treat musculoskeletal conditions. Antispasticity drugs like dantrolene are used to relieve muscle hypertonicity. These Antispasmodic and Antispasticity drugs have a lot of side effects this make them to use with caution.[5] Modern lifestyle has isolated us from the natural way of life. Since we have ignoring the rich natural heritage inherited from our ancestors, this ignorance has aggravated health issues such as digestive problems, aging problems and many more. Several researches are now being directed to explore natural herbs for their nutraceuticals, antimicrobial and nutritive potential. Many herbs are there which are of importance some of them are *P. attenuatum* (B. Ham), *Caesalpinia Crista* (Linn) and many more.

Branches of *P. attenuatum* (B.Ham.) are flexuous and glabrous. Leaves are 6.3 to 15 cm, often as broad as long, from finely downy to glabrous beneath, membranous, orbicular-ovate or cordate, abruptly acuminate, upper more ovate glabrous or puberulous beneath, 7- nerved from the base; base usually equally rounded, truncate or cordate, of the upper usually acute; nerves are slender; petiole 2.5–7.5 cm., rarely shorter.[6] Various phytoconstituents reported in *P. attenuatum* (B. Ham) are alkaloids like cepharadione A, cepharadione B, cepharanone B, guineensine, norcepharadione B, piperadione, piperine, piperlonguminine, Piperolactam A lignan like galbelgin, neolignans like Kadsurin A, Kadsurin B terpenes like β Bisabolene steroids like Sitosterol and miscellaneous compounds like Crotepoxide. Pipoxide chlorohydrin, Tetratriacontanoic acid.[7] Phytoconstituents present in *P. attenuatum* (B. Ham) showed strong potentiating effect on hexobarbital induced hypnosis and anti-convulsant in mice.[8] Phytoconstituents also causes decrease in body temperature,[9] CNS depressant activity and skeletal muscle relaxant effect.[8] There is no scientific information on Muscle relaxant and antioxidant effect of leaf extract of the plant *P. attenuatum* (B.Ham.). The aim of present study is to evaluate the muscle relaxant effect and antioxidant potential of leaf extract of the plant *P. attenuatum* (B.Ham.) scientifically to justify the traditional use of this plant.

MATERIALS AND METHODS

Plant Material

Fresh leaves of Plant *P. attenuatum* (B. Ham) was procured from Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) Palode, Thiruvananthapuram, Kerala, India. Plant was authenticated by Dr. Mathew Dan, Senior Scientist, Plant Genetic Resource division, JNTBGRI, Palode, Thiruvananthapuram, Kerala, India.

Preparetion of Ethanolic and Aqueous Leaf Extracts and Determination of %Yield

Authenticated leaves were washed with clean water before drying in oven at a temperature of 40℃ until the moisture content was below 14%. This reduces the chances of fungus infection in samples. The dried leaves were grinded and stored in airtight container.[10] Ethanolic extraction of plant leaf was carried out by Soxhlet extraction. Quantity of powdered material used for ethanolic extraction was 150 gm. This powdered material of plant leaf was defatted 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. Make the extracts dry under reduced pressure and controlled temperature (40-50°C) using flash evaporator.[11] Aqueous extract of plant leaf was prepared by simpal maceration process.[12] The total quantity of plant leaf for simpal maceration process was 200 gm. The ethanolic and aqueous extract obtained were concentrated under reduced pressure and % yield of both extracts were measured by using formula.

$$\text{Yield (\%)} = \frac{W_2 - W_1}{W_0} \times 100$$

Where

W_2 – Weight of extract and container

W_1 – Container weight

W_0 – initial dried sample weight[13]

Animals

Healthy Wistar albino rats and mice of both sexes weighing between 150 to 200 g and 30 to 40 g were taken for the study. They were housed under controlled conditions of temperature (22 ± 3℃), the relative humidity should be at least 30% but not exceed 70% (other than during room cleaning). It was 55 ± 5%. Lighting was artificial it was 12 hours light and 12 hours dark cycles according to OECD Guideline 423.Standard pellet diet and water were given to all animals. Approval of the Institutional Animals Ethics Committee was taken.

Chemical

DPPH, Hydrogen Peroxide, Distilled water, Ascorbic Acid purchased by SD Fine Chemicals Ltd. Diazepam (Lupin Laboratories Ltd., India), Gum acacia (Himedia Lab, India), and all other chemicals used in the study were of analytical grade and procured from local suppliers.

Phytochemical Characterization

Both ethanolic and aqueous extracts were subjected to general phytochemical analysis for the presence of carbohydrates, proteins, amino acids, tannins, phenolic,

flavonoids, alkaloids, anthraquinone, glycosides, saponins, and steroidal nucleus using the standard methods.[14-16]

Acute Toxicity Study and Dose Selection for Animal Screening

A total of three 8-12 week old non-pregnant female rats were used for the determination of Acute Toxicity. The animals were food-deprived overnight but free access to water prior to the experiment. The procedure was followed as per the OECD 423 guidelines. Ethanolic and aqueous extracts were administered orally in four doses: 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg orally. Animals were observed individually after first dose at least once during the first 30 minutes then periodically for the first 24 hours, giving special attention for first 4 hours and daily for a total of 14 days.[17] Following parameter were also observed alertness, irritability, Fearfulness, Touch response, Pain response, Restlessness, Righting reflex, Limb tone, Grip strength, Twitching, Abdominal tone, Pinna reflex, Corneal reflex, Tremors, Convulsions, Writhing, Defecation, Urination, Heart rate, Respiratory, Pupil size and Skin color.

Anti–Oxidant Activity

Reactive oxygen species (ROS) are responsible for damaging cellular biomolecules such as proteins, enzymes, nucleic acids, lipids and carbohydrates and may adversely affect immune functions. Antioxidants interrupt the production of ROS and also play a key role in inactivating them.[4]

In vitro Methods

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The evaluation of the antioxidant activity of ethanolic and aqueous leaf extracts of *P. attenuatum* (B. Ham) was conducted using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay. A stock solution of the both plant extracts (100 mg/mL) was diluted for 5 concentrations. The following concentrations of both extracts were prepared 10, 20, 30, 40 and 50 µg/mL. The portion of sample solution (0.5 mL) was mixed with 3.0ml of 0.1mM 1, 1-Diphenyl-2-2picrylhydrazyl (DPPH, in 95% distilled ethanol) and allowed to stand at room temperature for 30 minutes under light protection. The absorbance was measured at 517 nm. The scavenging activity of the samples at corresponded reaction mixture indicates higher free radical scavenging activity. The difference in absorbance between the sample and the control (DPPH in ethanol) was calculated and expressed as (%) scavenging of DPPH radical. In the DPPH test, antioxidants were typically characterized by their IC50 value (inhibition concentration of sample required to scavenge 50% of DPPH radicals).[3] The results were obtained by linear regression analysis of the dose-response curve plotted using % inhibition and concentration.

Scavenging of DPPH radical was calculated by using the following equation.

Scavenging activity (%) = [(Absorbance of Control- Absorbance of Sample)/(Absorbance of Control)] ×100[18]

Hydrogen Peroxide Free Radical Scavenging Assay

The ability of both extracts of *P. attenuatum* (B. Ham) to scavenge hydrogen peroxide was determined by H_2O_2 free radical scavenging assay. H_2O_2 solution (40 mM) was prepared in phosphate buffer (pH 7.4). A stock solution of both plant extracts (100mg/ml) was diluted for 5 concentrations. The following concentrations of both extracts were prepared 10, 20, 30, 40, and 50 µg/mL. Different concentrations of plant extracts were added to hydrogen peroxide solution (0.6 mL, 40 mM). H_2O_2 absorbance at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without H_2O_2. The percentage of hydrogen peroxide scavenging of both extracts and standard compounds (Ascorbic acid) were calculated.

$$\% \text{ Scavenged} = [(A_C - A_S)/A_C] \times 100$$

Where A_C is the absorbance of the control and A_S is the absorbance in the presence of the sample of *P. attenuatum* (B. Ham) extracts or standards.[19]

In Vivo Methods

Animals were divided into four groups containing six rats in each group. Group I was treated as control and received only normal saline. Groups II, III and IV received plant extract at a dose of 100, 200, and 300 mg/kg body weight, respectively. The treatments were carried out for 7 days and all the animals were sacrificed by cervical dislocation on 8th day. The liver removed and washed it with ice-cold saline, homogenization carried out in 10 volume of 0.1-M phosphate buffer (pH 7.4) containing 5-mM EDTA and 0.15 M NaCl centrifuged at 8000 rpm for 30 minutes at 4℃. The supernatant liquid was collected and used for the assay of enzyme activities.

Assay of Antioxidant Enzymes

Superoxide dismutase was assayed by measuring the inhibition of the formation of blue colored formazan at 560nm. Reduced glutathione level was measured spectrophotometrically at 412 nm.[4]

Skeletal Muscle Relaxant Activity using Rota Rod apparatus

The albino mice were divided into four groups of 6 each. The four groups were treated respectively as:

Group I-2 % gum acacia (10 mL/kg)

Group II-Diazepam (3 mg/kg)

Group III-Ethanolic Extract of Plant (100 mg/kg)

Group IV-Ethanolic Extract of Plant (200 mg/kg)

Balance and Motor coordination were tested using rotarod (Techno Rota Rod System). Mice were placed on a horizontal rod (3 cm diameter), rotating at an initial speed of 10 rpm/min. The velocity of the rod was linearly increased from 10 to 20 rpm within 20 seconds. The time to maintain balance, walking on top of the rod by animals was measured. Before the starting of experiments, the riding ability of the animals on rotarod was checked. Mice were trained on the rotating rod at 25 rounds/minute until they could balance to keep themselves rotating rod or till 60 seconds. 3 to 4 trials per day were given for 2 days to all the animals. Those mice that successively completed three trials/day for 2 days were selected. Mice that immediately dropped off or within 30 seconds were removed from the experiment. The number of seconds each mouse remained on the rotarod was recorded for 60 seconds on the third day.[20]

Statistical Analysis

Results interpretation was done after subjecting the data obtained from various studies. Statistical analysis was performed using Graph pad Prism 9.0.2 version which included one way ANOVA followed by test like Dunnett and t test. $P < 0.05$ is considered as statistically significant.

RESULT AND DISCUSSION

Yield (%) of Extracts and Phytochemical Screening

The % yield of ethanolic and aqueous leaf extracts of plant *P. attenuatum* (B. Ham) were (28.33%), (9.20%), respectively. The preliminary phytochemical analysis of ethanolic and aqueous leaf extracts of *P. attenuatum (B. Ham)* showed the presence of alkaloids, Tannin, flavonoids, steroids, and absence of Saponins results were given in Table 1.

Acute Toxicity Study and Dose Selection

Acute toxicity study was evaluated by using OECD guideline 423 on 8-12 week non-pregnant female rats. Various parameters like alertness, irritability, touch & pain response etc., are observed in rats during the observation period. The ethanolic and aqueous leaf extracts of *P. attenuatum* (B.Ham) was found to be non-toxic up to 2000mg/kg and did not cause any death Up to 5000 mg/kg. There was no mortality and behavior change in female rats. Following parameters were observed for 14 hours given in Table 2.

Table 1: Phytochemical screening of Plant *P. attenuatum* (B. Ham)

Sr. no	*Test*	*Observation*	*Aqueous extract*	*Ethanolic extract*
1.	Test for alkaloids Hager's Test Mayer's	All test were positive	+	+
2.	Test for steroids Salkowski reaction Liebermann-Burchard	All test were positive	+	+
3.	Test for flavonoids Ferric Chloride test Lead acetate test Alkaline solution	All test were positive	+	+
4.	Test for tannins Lead acetate test 5% Fe Cl_3 test	All test were positive	+	+
5.	Test for cardiac glycoside Legal's test Libermann test	All test were positive	+	+
6.	Solubility	–	In Water	In ethanol
7.	Test for saponin's Foam test	Negative	–	–

Antioxidant Activity

***In vitro* Methods**

- *DPPH Free Radical Scavenging Assay*

DPPH and H_2O_2 free radical scavenging activity of both ethanolic and aqueous leaf extract of plant *P. attenuatum* (B. Ham) at different concentration were given in Tables 1 and 2. Ascorbic acid was used as a standard in both assays.

DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical. DPPH is relatively stable nitrogen-free radical that can accept hydrogen or electron to become a stable diamagnetic molecule. DPPH radicals react with a reducing agent as a result of which electron become paired off and form corresponding hydrazine. Therefore, the solution loses color stoichiometrically depending on the number of electrons consumed, which is measured spectrometrically at 517 nm.[21] The mean IC_{50} value for DPPH radical of ascorbic acid was found to be 23 µg/mL. The mean IC_{50} value of ethanolic and aqueous extract was found to be 47 (µg/mL) and 49 (µg/ml), respectively. IC_{50} values are inversely related to the antioxidant activity; ascorbic acid has less IC_{50} value than ethanolic and aqueous extract; thus ascorbic acid has more DPPH free radical scavenging activity than ethanolic and aqueous extract.

Hydrogen Peroxide Free Radical Scavenging Assay

H_2O_2 is a weak oxidizing agent and can inactivate some enzymes directly, usually by oxidation of essential thiol (- SH) groups. Hydrogen peroxide can cross the cell membrane, inside the cell, H_2O_2 can react with Fe^{+2} and Cu^{+2} to form hydroxyl radicals, which may lead to many toxic effects. It is biologically advantageous for cells to control the amount of H_2O_2 that is allowed to accumulate. The decomposition of H_2O_2 by extracts of *Glycosmis pentaphylla* may result from its antioxidant and free radical scavenging activity.[21]

The mean IC_{50} value to ascorbic acid, ethanolic and aqueous extract of plant *P. attenuatum* (B. Ham) was 16 mcg/ml, 43 mcg/mL and 47mcg/mL, respectively. %scavenging activity of plant extract and standard is given in Table 4.

Table 3 and 4 showed that ethanolic and aqueous leaf extracts of plant *P. attenuatum* (B. Ham) showed significant antioxidant activity of Plant *P. attenuatum* (B. Ham).

***In Vivo* Methods**

Assay of Antioxidant Enzymes (SOD, GSH)

Both ethanolic and aqueous leaf extracts of plant *P. attenuatum* (B. Ham) at a dose of 100, 200, 300 mg/kg were given to rats for 7 days; this significantly enhance

Table 2: Acute oral toxicity study of different extracts of plant *P. attenuatum* (B. Ham)

	Alertness	*Irritability*	*Fearfulness*	*Touch response*	*Pain response*	*Grooming*	*Restlessness*	*Limb tone*	*Grip strength*	*Abdominal tone*	*Pinna reflex*	*Corneal reflux*	*Tremor*	*Convulsion*	*Writhing*	*Defecation*	*Urination*	*Heart rate*	*Respiratory rate*	*Pupil size*	*Skin colour*
Ethanolic Extract	N	_	_	N	N	_	_	N	N	N	N	N	_	_	N	N	N	N	N	N	N
Aqueous Extract	N	_	_	N	N	_	_	N	N	N	N	N	_	_	N	N	N	N	N	N	N

(N) = Normal, (-) = Not Present, (+) = Present

Table 3: DPPH free radical scavenging activity of ethanolic & aqueous extract of different concentration

	DPPH free radical scavenging activity (Mean ± SEM) of ethanolic & aqueous extracts of different concentration					
Sample	*10µg/ml*	*20 µg/ml*	*30 µg/ml*	*40 µg/ml*	*50 µg/ml*	*IC_{50}*
Ethanolic extract(A)	10.15 ± 0.01*	12.23 ± 0.05*	20.76 ± 0.04*	28.30 ± 0.03*	58.69 ± 0.04*	47
Aqueous extract (B)	12.15 ± 0.07*	13.00 ± 0.06 *	22.30 ± 0.04 *	30.61 ± 0.02 *	55.15 ± 0.01 *	49
Ascorbic acid (C)	25.73 ± 0.08	45.64 ± 0.09	77.64 ± 0.12	89.64 ± 0.127	96.09 ± 0.26	23

p Value summary * p < .05, ** p < .01, *** p <.001 When compared with Standard
p-value for A-C = 0.0404
p-value for B-C = 0.0445

Table 4: H_2O_2 Free radical scavenging activity of ethanolic and aqueous extract of different concentration

	H_2O_2 free radical scavenging activity (mean ± SEM) of ethanolic and aqueous extracts of different concentration					
Sample	*10 µg/mL*	*20 µg/mL*	*30 µg/mL*	*40 µg/mL*	*50 µg/mL*	*IC_{50}*
Ethanolic extract (A)	18.12 ± 0.43*	31.24 ± 1.19*	31.21 ± 3.24*	47.43 ± 2.53*	59.37 ± 2.47*	43
Aqueous extract (B)	20.38 ± 0.59*	28.63 ± 1.93*	30.75 ± 2.38*	43.49 ± 1.29*	50.08 ± 2.98*	47
Ascorbic Acid (C)	30.73 ± 0.83	61.85 ± 1.21	74.19 ± 1.82	82.89 ± 1.98	90.18 ± 2.00	16

p-value summary * p < .05, ** p < .01, *** p <.001When compared with Standard
p-value for A-C = 0.0489
p-value for B-C = 0.0305

Table 5: Determination of SOD and GSH Anti-oxidant enzyme level in ethanolic extract-treated rats

Sr. No	*Tested parameter*	*Group I (normal saline)*	*Group II (100 mg/kg Ethanolic extract)*	*Group III (200 mg/kg Ethanolic extract)*	*Group IV (300 mg/kg Ethanolic extract)*
1.	SOD	1.25 ± 0.54	2.60 ± 0.2**	2.98 ± 0.19***	3.32 ± 0.2***
2.	GSH	1.18 ± 0.04	1.45 ± 0.12*	1.78 ± 0.08**	2.5 ± 0.09***

Table 6: Determination of SOD & GSH Anti-oxidant enzyme level in aqueous extract treated rats

Sr. No	*Tested parameter*	*Group I (Normal saline)*	*Group II (100 mg/kg Aqueous extract)*	*Group III (200 mg/kg Aqueous extract)*	*Group IV (300 mg/kg Aqueous extract)*
1.	SOD	1.25 ± 0.54	1.55 ± 0.2**	1.60 ± 0.19***	2.37 ± 0.2***
2.	GSH	1.18 ± 0.04	1.45 ± 0.12*	1.58 ± 0.08*	2.5 ± 0.09***

p <0.01 for SOD at 100mg/kg aqueous dose and p <0.001 for SOD at 200 mg/kg and 300 mg/kg aqueous dose. p <0.05 for GSH at 100mg/kg and 200mg/kg aqueous dose, p <0.001 for GSH at 300 mg/kg aqueous dose.

Superoxide dismutase and glutathione level when compared to control group (Group I).

Both extracts showed significant results, p <0.01 for SOD at 100 mg/kg ethanolic dose and p <0.001 for SOD at 200 mg/kg and 300 mg/kg ethanolic dose. p <0.05 for GSH at 100mg/kg ethanolic dose, p <0.01 for GSH at 200 mg/kg ethanolic dose and p <0.001 for GSH at 300 mg/kg ethanolic dose.

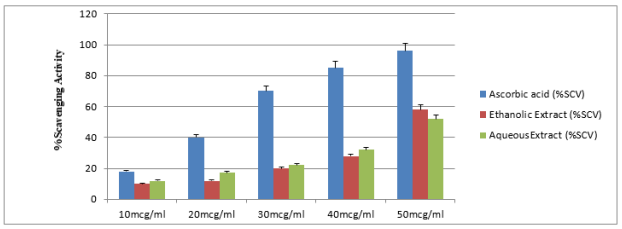

Figure 1: DPPH scavenging activity of different concentrations of Plant Extracts

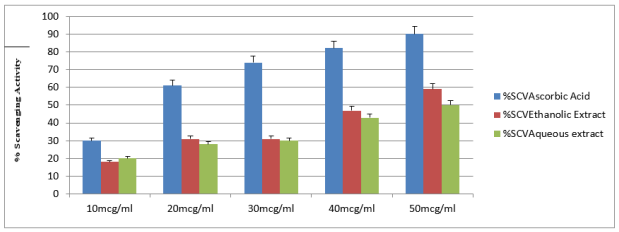

Figure 2: H_2O_2 free radical scavenging activity of different concentrations of Plant Extracts

Skeletal Muscle Relaxant Activity Using Rota Rod Apparatus

Plant extract 200 mg/kg showed significant reduction in the mice's time on the revolving rod compared to the control ($p < 0.05$). The standard drug (diazepam) showed a highly significant effect when compared to the control ($p < 0.0001$). However, Maximum muscle relaxation was observed with 200 mg/kg of ethanolic extract of *P. attenuatum* (B.Ham) while muscle relaxant effect of plant at dose 100 mg/kg was not significant when compared to control group (p-value 0.9925). The Rotarod test result showed that the extract at dose 200 mg/kg significantly reduced the motor coordination of the tested animals.

Piperine which is an alkaloid found in almost all piper species, also presents in ethanolic and aqueous leaf extract of plant *P. attenuatum* (B. Ham).[7] Piperine is responsible

Table 7: Skeletal muscle relaxant activity using rotarod apparatus, One way ANOVA followed by multiple tukey's comparison test.

Sr. No	*Treatment Group*	*Dose mg/kg Orally*	*Basal Reading in seconds*	*Mean time on Rotarod after treatment*			
				60 sec	*90 sec*	*120 sec*	*240sec*
1.	Control	10ml/kg of 2% gum acacia	60	57.16 ± 1.13	55.83 ± 1.71	56.16 ± 1.81	57.33 ± 0.83
2.	Standard	3mg/kg	60	40.83 ± 1.98****	42.66 ± 1.76****	45.33 ± 1.68****	50.33 ± 2.20****
3.	Ethanolic Extract	100mg/kg	60	54.16 ± 1.34^{NS}	55.50 ± 1.60^{NS}	56.16 ± 1.06^{NS}	58.83 ± 0.68^{NS}
4.	Ethanolic Extract	200mg/kg	60	49.83 ± 1.98*	50.83 ± 2.39*	51.5 ± 1.78*	52 ± 2.67*

Values are Mean ± SD, *p <0.05, ****p <.0001

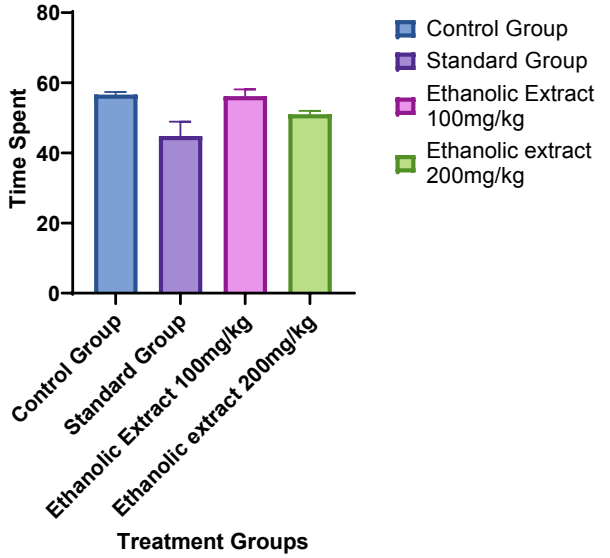

Figure 3: Muscle relaxant effect of Plant extracts (100 mg/kg and 200 mg/kg) by using Rotarod apparatus,
Values are Mean ± SD, *P <0.05, ****P <.0001

for muscle relaxation activity as mentioned in the traditional Indian system of medicine.[8]

CONCLUSION

Present study revealed that both ethanolic and aqueous extract of authenticated leaf part of plant *P. attenuatum* (B. Ham) showed the presence of various phytoconstituents like alkaloids, tannins, flavonoids etc. both the extract having antioxidant activity at various concentrations, while ethanolic extract showed significant muscle relaxant activity at 200 mg/kg dose. Acute toxicity study was also performed on female rats which showed that both extracts are non-toxic Upto 2000 mg/kg and did not cause any death Upto 5000 mg/kg dose.

REFERENCES

1. Maurya U, Srivastava S. Traditional Indian herbal medicine used as antipyretic, antiulcer, anti-diabetic and anticancer. International Journal of Research in Pharmacy and Chemistry. 2011;1(4): 1152-9.
2. Samy RP, Pushparaj PN, Gopalakrishnakone P. A compilation of bioactive compounds from Ayurveda. Bioinformation. 2008;3(3):100-10.
3. Nahak G, Sahu RK. Phytochemical evaluation and antioxidant activity of *Piper cubeba* and *Piper nigrum*. Journal of applied Pharmaceutical Sciences. 2011;1(8):153-7.
4. Mandal S, Hazra B, Sarkar R, Biswas S, Mandal N. Assessment of the Antioxidant and Reactive Oxygen Species Scavenging Activity of Methanolic Extract of *Caesalpinia crista* leaf. Evidence-based Complementary and Alternative Medicine. 2011:1-11. Available from: doi: 10.1093/ecam/nep072
5. Shetty AK, Vinutha N, Udasimath SF, Kalleshappa CM, Krupanidhi AM, Akshara. Evaluation of Skeletal Muscle Relaxant Activity Of Ethanol Extract of *Chromoleana Odorata*. IOSR Journal of Pharmacy. 2017;7(3):2319-4219.
6. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nded. Dehradun: International book distributor; 2006.
7. Parmar VS, Jain SC, Bisht KS, Jain R, Taneja P, Jha A et. al. Phytochemistry of the genus piper. Pergamon 1997;46(4):591-673.
8. Kirtikar KR, Basu BD. Compendium of Indian medicinal plants. 2nded. Dehradun: International book distributor; 2006.
9. Soni G, Verma T, Rajora M. Anti-pyretic activity of seed extract of Plant *Piper attenuatum* (B. Ham) in experimental animals. Inventi rapid Ethnopharmacol. 2014; 2014(2): 1-4.
10. Pin KY, Chuah TG, Rashih AA, Rasadah MA, Law CL, Choong TSY. Aqueous extraction of Hydroxychavicol from *Piper betel* L. leaves. Proceedings of the 1st International

Conference on Natural Resources Engineering & Technology. 2006; Kepong, Selangor, Putrajaya, Malaysia.

11. Soni G, Verma T, Verma D. Screening of ethanolic seed extract of *Piper attenuatum* (B. Ham) in Streptozotocin induced type 2 diabetes in rats. Inventi rapid Ethnopharmacol. 2014;2014(2):1-4.
12. Sharma RK, Mandal S, Rajni GP, Gupta N, Srivastava DP. Anti-ulcer and anti-inflammatory activity of fresh leaves extract of *Polyalthia Longifolia* in rats. International Journal of Drug Development and Research. 2011;3(1):351-9.
13. Syahidah A, Saad CR, Hassan MD, Rukayadi Y, Norazian MH, Kamarudin MS. Phytochemical analysis, identification and quantification of antibacterial active compounds in betel leaves, *Piper betel* methanolic extract. Pakistan Journal of Biological Sciences. 2017;20(2):70-81. Available from: DOI: 10.3923/pjbs.2017.70.81
14. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 24th ed. Pune: Nirali Prakashan; 2003.
15. Evans WC. Pharmacognosy. 15th ed. Newyork: Oxford Philadelphia; 2000.
16. Sahu VK, Irchhaiya R, Sashi A, Gurjar H. Phytochemical investigation and chromatographic evaluation of the ethanolic extract of whole plant extract of *dendrophthoe falcata* (l.f.) *Ettingsh*. International Journal of Pharmaceutical Science & Research. 2010;1(1):39-45. Available from: http://dx.doi.org/10.13040/IJPSR.0975-8232.1(1).39-45
17. OECD guidelines for the testing of chemicals (Acute oral toxicity - Acute toxic class method). Adopted 2001: Available from: URL: www.oecd.org.
18. Perumal P, Saravanabhavan K. Antidiabetic and antioxidant activities of ethanolic extract of *piper betle* Leaves in catfish, *clarias gariepinus*. Asian Journal of Pharmaceutical and Clinical Research. 2018;11(3):194-8. Available from: DOI: 10.22159/ajpcr.2018.v11i3.22393
19. Keser S, Celik S, Turkoglu S, Yilmez O, Turkoglu I. Hydrogen Peroxide radical scavenging and total anti oxidant activity hawthorn. Chemistry Journal. 2012;02(1):9-12.
20. Mishra PS, Phadnis P, Vyas S, Nyati P. Study of CNS activities of Piperine *Perse* and its Bio-Enhancing effect on various drugs in experimental animal models. International Journal of Pharmacy and Pharmaceutical Science. 2016;8(4): 135-40.
21. Gupta N, Agarwal M, Bhatia V, Jha SK, Dinesh J. Invitro antioxidant activity of crude extracts of plant *Glycosmis pentaphylla Correa*. International Journal of Pharmaceutical Sciences Review and Research. 2011;6(2):159-62.