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Evaluation of *in vitro* anti snake venom activity of *Justicia adhatoda* leaves extract against Russell's viper snake venom

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ABSTRACT

Venomous snake bites possess significant amount of mortality as well as morbidity in tropical and subtropical regions of the world including India. The snake Russell's viper (*Daboia russelii*) is responsible for thousands of deaths in India. Although antivenom are the only treatment available and the effects associated with several side effects. As an alternative, plants have been extensively studied in order to obtain an alternative treatment. The uses of plants against the effects of snake bites have been long recognized more scientific attention has been given since last 20 years. Extracts from plants have been used among traditional healers, especially in tropical areas there are plentiful sources, as therapy for snakebite for a long time. The present study aimed to provide a scientific explanation for the use of *Justicia adhatoda* plant against snakebite. The acetone extract of *Justicia adhatoda* leaves were tested for *in vitro* inhibitory activity on toxic venom enzymes like 5' nucleotidase, L-amino acid oxidase, phosphomonoesterase, phosphodiesterase, acetylcholine esterase and hyaluronidase from the Russell's viper snake venom. The result showed the potential of plant leaves extract against the enzymes present in the snake venom. *Justicia adhatoda* inhibits most of the snake venom enzymes like L-amino acid oxidase, phosphomonoesterase, phosphodiestarase and acetylcholine esterase.

Keywords: *Daboia russelii*, Enzyme inhibition, Acetone Extract of *Justicia adhatoda* (AEJA), etc.

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1. INTRODUCTION

Venomous snakebites represent a severe medical, social, and economic challenge in many parts of the world, particularly in tropical and subtropical nations. Majority of the world's dangerous snakes are found in developing countries. Population in these regions particularly in Africa, south Asia, Latin America, and parts of Oceania experience high mortality because of poor access to health services. Conservative data indicated that, worldwide, there are between 2.5 to 5.4 million snakebites are recorded in every year, leading to 125,000 to 150,000 deaths [1]. The snake envenomation is the major problem which is included in the list of 'Neglected Tropical Diseases' since 2009 by World Health Organization[2].

The Russell's viper is considered as a highly venomous snake throughout their range in south Asia including India, Pakistan, Bangladesh, Sri Lanka, Myanmar, Thailand, Taiwan and Indonesia. Snake venoms are complex mixture of some enzymatic and toxic proteins, which include phospholipase A_2 (PLA_2s), myotoxins, hemorrhagic metalloproteinases and other proteolytic enzymes, coagulant components, cardiotoxins, cytotoxins and neurotoxins[3]. Russell's viper envenomation includes pain, swelling, myonecrosis and renal failure. The antiserum is the only therapeutic agent available for the treatment of snakebite and does not provide enough protection against venom inducing haemorrhage, necrosis, and nephrotoxicity often produces hypersensitive reactions.

Antiserum development in animal is time consuming, very expensive and requires ideal storage condition. Monovalent antiserum is not available in many regions and the health center is usually far and few in number. To overcome these drawbacks, there is a great need to search, to develop new affordable and suitable antidote against snakebite. In India, polyvalent antivenom is raised against the "Big four" snake venoms (namely *Daboia russelii*, *Naja naja*, *Echis carinatus* and *Bungarus caeruleus*) which is the only available treatment for snake envenomation. However, during the time, administration of antivenom is accompanied by some anaphylactic reactions like nausea, vomiting, hypotension, respiratory discomfort and low body temperature [4].

Plants have played a critical role in maintaining human health and civilizing the quality of human life for thousands of years. The use of plants as medicines is as old as human civilization itself and out of about 258,650 species of higher plants reported from the world and more than 10% are used to cure ailing many communities [5]. The anti-snake venom plants contain secondary metabolites that are responsible for venom neutralization. Plants are used either single or in combination, as antidotes for snake envenomation by rural populations in India and in many parts of the world. In most developing countries, up to 80% of individuals bitten by snakes first consult traditional practitioners before visiting a medical centre. Traditional herbal medicine is readily available in rural areas for the treatment of snakebite. Even today, indigenous and certain local communities practice herbal medicine to cure a variety of diseases, with plants particularly used as folk medicine to treat snakebites [6].

The genus *Justicia* showed extraordinary activity of antisnake venom ability from the traditional healers used in snakebite in the southern parts of India [7]. The bruised fresh leaves used for snakebite in India and Srilanka. The leaves and the root were used for medicinal purpose. By this reason here this plant were used to investigate the neutralization of lethal effects of the *Daboia russelii* snake venom.

2. MATERIALS AND METHODS

2.1 Collection and authentication of plant material

The fresh leaves of *Justicia adhatoda* plant was collected from the Kurumbalur village, Perambalur district, Tamilnadu, India during the month of October-November 2017. The plant specimen were identified and authenticated by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Trichy, Tamilnadu (Authentication number: DKA 001). The leaves were thoroughly washed in order to remove adhering dust, shade dried under room temperature for 7-8 days. The dried plant leaves were powdered in a mixer grinder and stored for further use.

2.2 Extract preparation

The extract was prepared by adding 20g of dried and powdered leaves were separately mounted on the Soxhlet extractor and extracted with 100 ml of solvent acetone. The extraction procedure was carried out until the solvent becomes colorless in the Soxhlet loop. Then extract were concentrated by keeping them at room temperature to allow the solvent to evaporate and the residue was expressed in terms of dry weight, which was used for further analysis. Then the extracts was stored in an air tight container then refrigerated at 4˚C for further use.

2.3 Collection of venom

The crude venom was extracted from the Russell's viper snake captured by Irula tribal of Kurumbalur village, Perambalur district. The snake venom was extracted by Milking process [8]. The crude venom was dissolved in phosphate buffer and centrifuged at 2000 rpm for ten minutes. The supernatant was used for further analysis and stored at 4˚C.

2.4 *In vitro* snake venom inhibition studies

2.4.1 Inhibition of 5' Nucleotidase enzyme activity

The 5' Nucleotidase enzyme activity was assayed by the method of Rowe *et al.*[9]. The substrate solution contained 1 ml of Tris-HCl buffer (pH 8.0), 0.1 ml of 0.1 M magnesium chloride and 0.8 ml of 0.15% 5'adenosine mono phosphate followed by various concentrations (0.25 ml to 1.25ml) of 0.1% crude venom and incubated at 37°C for 15 minutes. At the end of incubation time, the reaction was quenched by adding TCA and filtered. The filtrate was assayed for inorganic phosphate at 625 nm using potassium dihydrogen phosphate as standard. In this analysis, one unit of enzyme activity was defined as the amount that yielded 0.01 µmole of inorganic phosphate/minute under the experimental conditions. For the inhibition studies, venom was preincubated with various concentrations of (50µg to 300 µg) plant extract for 30 minutes at 37°C.

2.4.2 Inhibition of L-amino acid oxidase enzyme activity

The L-amino acid oxidase activity was carried out according to Li *et al.*[10]. Reaction mixture consisted of 1.0 ml of 0.1% L-leucine, 2.0 ml of Tris-HCl buffer (pH 8.0), 0.25 ml of 0.1% dianisidine hydrochloride, 0.15 ml of 0.1% horseradish peroxidase and various concentrations (0.04 ml to 0.20 ml) of 0.5% crude venom solution. It was allowed to stand for ten minutes at room temperature and then the absorbance was measured at 415 nm. One unit (U) was defined as the amount of enzyme that catalyzed the formation of 1 µmol H_2O_2 per minute. For the inhibition studies, venom was preincubated with various concentrations of (50µg to 300µg) plant extract for 30 minutes at 37°C.

2.4.3 Inhibition of Hyaluronidase enzyme activity

Hyaluronidase assay of crude venom was determined turbidometrically by the method of Pukrittayakamee *et al.*[11]. The assay mixture contained buffer Tris-HCl (pH 8.0), 0.5 ml of hyaluronic acid (0.5 mg/ml in buffer) and various concentrations (0.05 ml to 0.25ml) of venom solution. The mixture was incubated for 15 minutes at 37°C and the reaction was quenched by the addition of 2 ml of 2.5% (w/v) cetyl-trimethylammonium bromide in 2% NaOH (w/v). The absorbance was read at 400 nm (within ten minutes) against a control solution containing 1 ml of the same buffer and 2 ml of 2.5% (w/v) cetyl-trimethylammonium bromide in 2% NaOH (w/v). Turbidity reducing activity was expressed as a percentage of the remaining hyaluronic acid, taking the absorbance of a tube in which no enzyme was added as 100%. One unit was defined as the amount of enzyme that provoked 50% turbidity reduction. Specific activity was defined as turbidity reducing units per milligram of enzyme. For the inhibition studies, venom was preincubated with various concentrations of (50µg to 300µg) plant extract for 30 minutes at 37°C.

2.4.4 Inhibition of Phosphomonoesterase enzyme activity

The phosphomonoesterase activity was determined by the method of Bessey *et al.*[12] with slight modifications. The reaction mixture included 1 ml of Tris-HCl buffer (pH 8.0), 1.0 ml of 0.0025 M disodium-p-nitrophenol phosphate, various concentration (0.1ml to 0.5 ml) of 0.25% crude venom and was incubated at 37°C for three hours. The absorbance was measured at 425 nm. p-nitrophenol was used as the standard. One unit of enzyme activity was defined as the amount that yielded 0.1 µmole of p-nitrophenol/ hour under the experimental conditions. For inhibition studies, venom was preincubated with various concentrations of (50µg to 300µg) plant extract for 30 minutes at 37°C.

2.4.5 Inhibition of Phosphodiesterase enzyme activity

Phosphodiesterase activity was determined by a method modified from Lo *et al.*[13]. The assay mixture contained various concentration (0.1 ml to 0.5ml) of venom solution, 0.5 ml of 0.0025 M Na-p-nitrophenyl phosphate, 0.3 ml of 0.01 M $MgSO_4$ and 0.5mL of 0.17M Tris-HCl (pH 8.0) and was

incubated at 37°C for three hours. The absorbance was measured at 400 nm using UV Spectrophotometer. Phosphodiesterase activity was expressed in nanomoles of product released/minute. Molar extinction coefficient at 400 nm was 8100 Cm^{-1} M^{-1}. For the inhibition studies, the venom was preincubated with various concentrations of (50µg to 300µg) plant extracts for 30 minutes at 37°C.

2.4.6 Inhibition of Acetylcholine esterase enzyme activity

Acetylcholine esterase activity was assayed by the method of Ellman *et al.*[14] method. The reaction mixture comprised 3.0 ml of the phosphate buffer (pH 8.0), 10 µl of DTNB (10 mmole/l) and 20 µl of acetylethiocholine iodide (158.5 mmol/l). A total of 50 µl of 0.1% crude venom and 3 ml of buffer solution were incubated at room temperature for five minutes. Then, 10 µl of 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) (a strong oxidizing agent) and 20 µl of substrate acetylethiocholine iodide were added in order to reach a final concentration of 1 mmole/l. The increase in absorbance at 412 nm was measured on double beam spectrophotometer against control mixture prepared at the same time. However, in the latter case, 50 µl of enzyme was replaced with 50 µl of buffer solution. For the inhibition studies, venom was preincubated with various concentration of (50µg to 300µg) plant extract for 30 minutes at 37°C.

2.5 Statistical analysis

Results obtained were reported as mean ± SD of triplicate measurements. Significance differences for multiple comparisons were determined by One-way ANOVA with $p<0.005$ using SPSS (version 19).

3. RESULTS

3.1 Results of enzyme inhibition studies

The acetone extract of *Justicia adhatoda* leaves showed the significant ($p<0.005$) enzyme inhibitory activity against the Russell's viper venom. Enzymes are the major components of the snake venom which is responsible for the lethality. In the present study, the acetone extract of *Justicia adhatoda* leaves was tested against six enzymes that mainly present in the Russell's viper venom. The extract was tested with six different concentrations i.e., 50µg to 300µg. The inhibitory activity was observed in dose dependent manner. The acetone extract of *Justicia adhatoda* leaves was significantly ($p<0.005$) decreased the enzyme activity when the extract concentration was increased.

3.1.1 Inhibition of 5' Nucleotidase enzyme activity

The extract of leaves was assessed for the inhibitory effects against the 5'Nucleotidase enzyme activity present in Russell's viper snake venom. The Acetone Extract of *Justicia adhatoda* (AEJA) was showed a significant ($p<0.005$) inhibitory activity against the 5'Nucleotidase enzyme (figure 1). The AEJA was treated with six different concentrations (50µg - 300µg). The venom was served alone in six different concentrations (0.25ml to 1.50ml) to test the efficacy of the enzyme. Among them the 1.25ml concentration of venom was showed the maximum enzyme activity (figure 1). Hence the 1.25ml concentration was selected as optimum dose for inhibition studies. The 1.25ml of snake venom was preincubated for 30 minutes at 37˚C with six different concentrations (50µg to 300µg) of acetone extract of *Justicia adhatoda* leaves for inhibition studies.

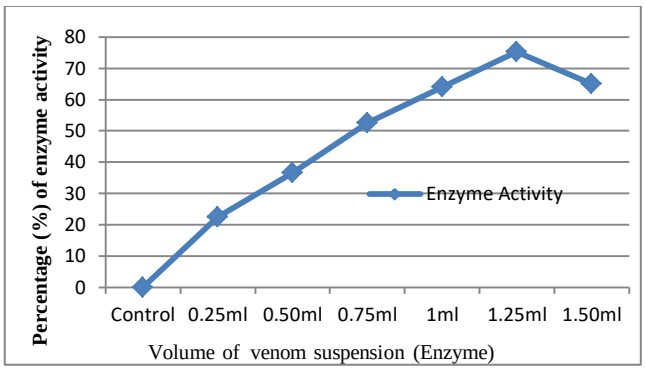

Figure 1: Efficacy of 5'Nucleotidase enzyme activity

In inhibition study, the AEJA was significantly ($p<0.005$) decreased the enzyme activity by dose dependant manner up to 250µg concentration when compared to the venom served alone. The maximum enzyme inhibitory effect was observed at 250µg concentration of AEJA. This clearly indicates that the AEJA was significantly ($p<0.005$) inhibited the 5'Nucleotidase enzyme activity (figure 1a). The observed result was statistically significant ($p<0.005$) when compared to the control.

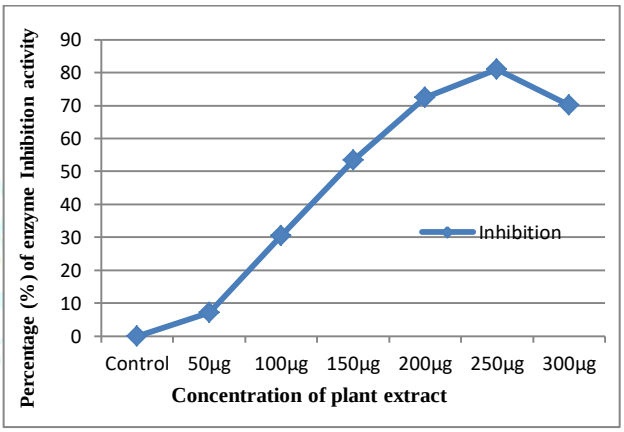

Figure 1a: Inhibition of 5'Nucleotidase enzyme activity

3.1.2 Inhibition of L-amino acid oxidase enzyme activity

The acetone extract of *Justicia adhatoda* leaves was tested for the effectiveness against the L-Amino acid oxidase enzyme inhibition activity. The venom was served alone at six different concentrations (0.04ml to 0.24ml) to test the effectiveness of the L-Amino acid oxidase enzyme present in the snake venom. Among all the tested concentrations, the 0.20ml concentration of snake venom showed a maximum enzyme activity (figure 2.).Hence the 0.20ml concentration of snake venom was selected as optimum dose for the enzyme inhibition study.

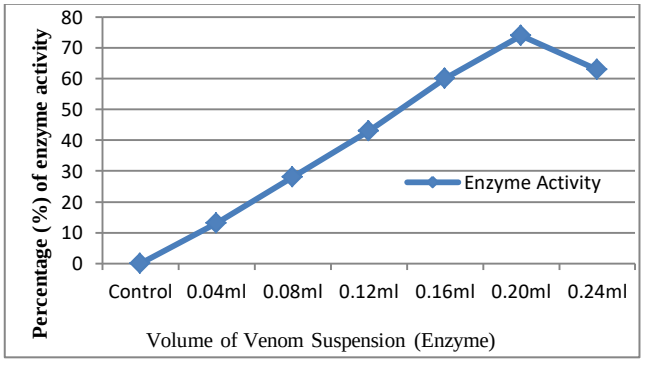

Figure 2: Efficacy of L-amino acid oxidase enzyme activity

In inhibition study, the 0.20ml of snake venom was preincubated for 30 minutes at 37˚C with six different concentrations (50µg to 300µg) of acetone extract of *Justicia adhatoda* (AEJA) leaves. In inhibition study, the AEJA was significantly (p<0.005) decreased the enzyme activity by dose dependant manner up to 250µg concentration when compared to the venom served alone. The maximum enzyme inhibition activity was observed at 250µg concentration of acetone extract of *Justicia adhatoda* leaves. This result was clearly demonstrates that the AEJA was significantly (p<0.005) inhibited the L-Amino acid oxidase enzyme activity (figure 2a). The observed result was statistically significant (p<0.005) to the control.

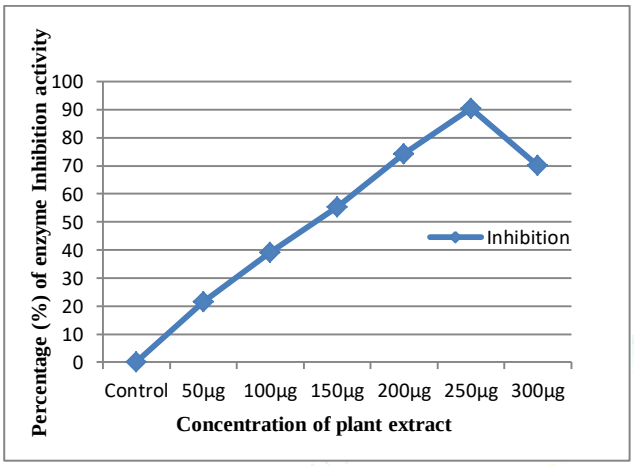

Figure 2a: Inhibition of L-amino acid oxidase enzyme activity

3.1.3 Inhibition of Hyaluronidase Enzyme activity

The acetone extract of *Justicia adhatoda* leaves was tested for the effectiveness against the hyaluronidase enzyme inhibition activity. The venom was previously served alone at six different concentrations (0.1ml to 0.6ml) to test the effectiveness of the hyaluronidase enzyme present in the snake venom. Among all the tested concentrations, the 0.5ml concentration of snake venom showed a maximum enzyme activity (figure 3). Hence the 0.5ml concentration of snake venom was selected as optimum dose for the enzyme inhibition study.

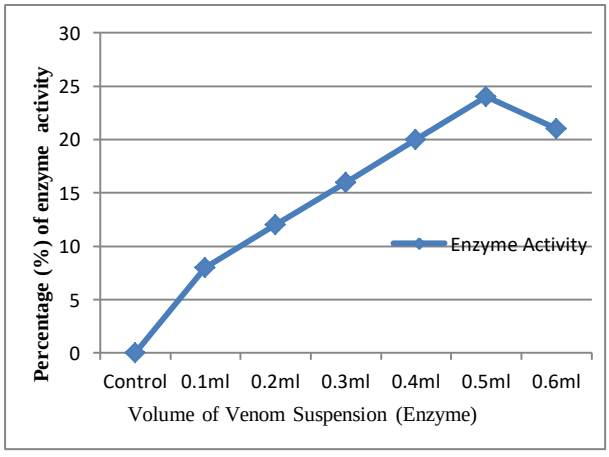

Figure 3: Efficacy of hyaluronidase enzyme activity

In inhibition study, the 0.5ml of snake venom was preincubated for 30 minutes at 37˚C with six different concentrations (50µg to 300µg) of acetone extract of *Justicia adhatoda* leaves. In inhibition study, the AEJA was significantly (p<0.005) decreased the enzyme activity by dose dependant manner up to 250µg concentration when compared to the venom served alone. The maximum enzyme inhibition activity was observed at 250µg concentration of acetone extract of *Justicia adhatoda* leaves. This result was clearly demonstrates that the AEJA was significantly (p<0.005) inhibited the hyaluronidase enzyme activity (figure.3a). The observed result was statistically significant (p<0.005) to the control.

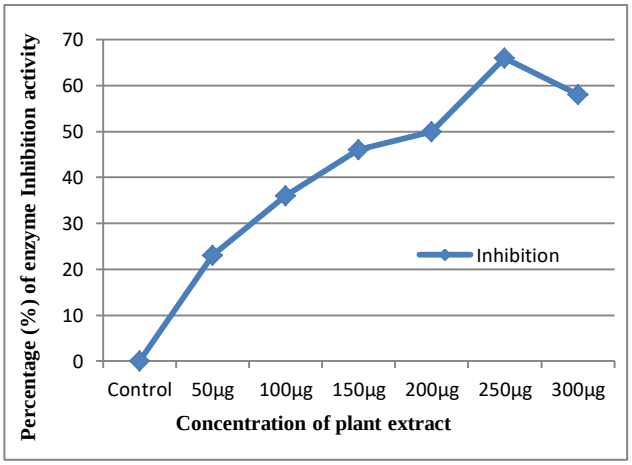

Figure 3a: Inhibition of Hyalurodinase enzyme activity

3.1.4 Inhibition of Phosphomonoesterase enzyme activity

The acetone extract of *Justicia adhatoda* leaves was tested against the phosphomonoesterase enzyme activity. The snake venom was served alone at six different concentrations (0.1ml to 0.6ml) to test the efficacy of the enzyme. Among the 0.5ml concentration of snake venom possesses the maximum enzyme activity when compared to the other concentrations (figure 4). Hence, the 0.5ml concentration of venom was selected as optimum dose for the enzyme inhibition study. In inhibition study, the 0.5ml of snake venom was preincubated for 30 minutes at 37˚C with eight different concentrations (50µg to 300µg) of acetone extract of *Justicia adhatoda* leaves.

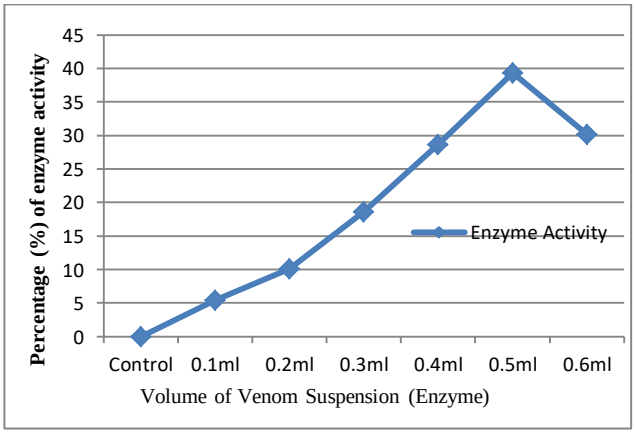

Figure 4: Efficacy of phosphomonoesterase enzyme activity

In inhibition study, the AEJA was significantly (p<0.005) decreased the enzyme activity by dose dependant manner up to 250µg concentration when compared to the venom served alone. The maximum enzyme inhibitory effect was observed at 250µg concentration of acetone extract of *Justicia adhatoda* leaves. This clearly indicates that the AEJA was significantly (p<0.005) inhibited the phosphomonoesterase enzyme activity (figure 4a). The observed result was statistically significant (p<0.005) when compared to the control.

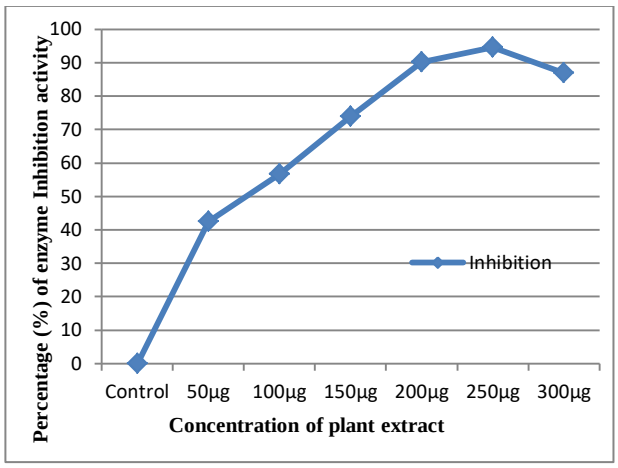

Figure 4a: Inhibition of phosphomonoestarase enzyme activity

3.1.5 Inhibition of Phosphodiesterase enzyme activity

The acetone extract of *Justicia adhatoda* leaves was tested against the phosphodiesterase enzyme activity. Previously the venom of Russell's viper was served alone at six different concentrations (0.1ml to 0.6ml) to test the effectiveness of the enzyme. Among the tested concentrations the 0.5ml concentration of venom showed a maximum enzyme activity (figure 5). For this reason, the 0.5ml concentration of snake venom was selected as optimum dose for the inhibition study.

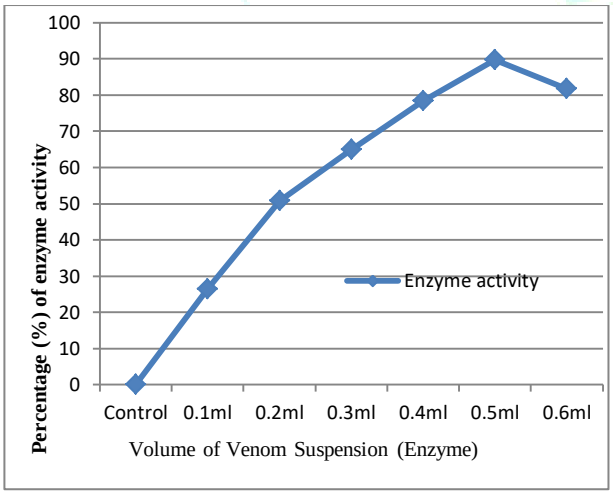

Figure 5: Efficacy of phophodiestarse enzyme activity

In inhibition study, the 0.5ml of snake venom was preincubated for 30 minutes at 37˚C with six different concentrations (50µg to 300µg) of acetone extract of *Justicia adhatoda* leaves. In inhibition study, the AEJA was significantly ($p<0.005$) decreased the enzyme activity by dose dependant manner up to 250µg concentration when compared to the venom served alone. The maximum enzyme inhibition activity was observed at 250µg concentration of acetone extract of *Justicia adhatoda* leaves. This result was clearly indicates that the AEJA was significantly ($p<0.005$) inhibited the phosphodiesterase enzyme activity (figure 5a). The observed result was statistically significant ($p<0.005$) when compared to the control.

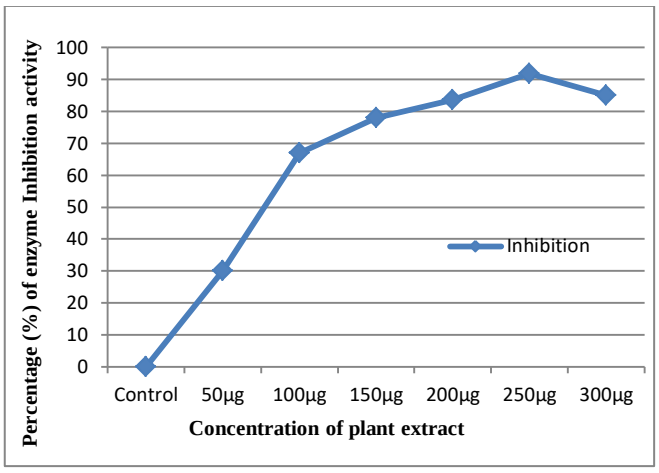

Figure 5a: Inhibition of phosphodiestarase enzyme activity

3.1.6 Inhibition of Acetylcholine esterase enzyme activity

The acetone extract of *Justicia adhatoda* leaves was tested for the effectiveness against the acetylcholine esterase enzyme inhibitory activity. The venom was previously served alone at six different concentrations (0.05ml to 0.30ml) to test the effectiveness of the Acetylcholine esterase enzyme present in the snake venom. Among all the tested concentrations, the 0.25ml concentration of snake venom showed a maximum enzyme activity (figure 6). Hence the 0.25ml concentration of snake venom was selected as optimum dose for the enzyme inhibition study.

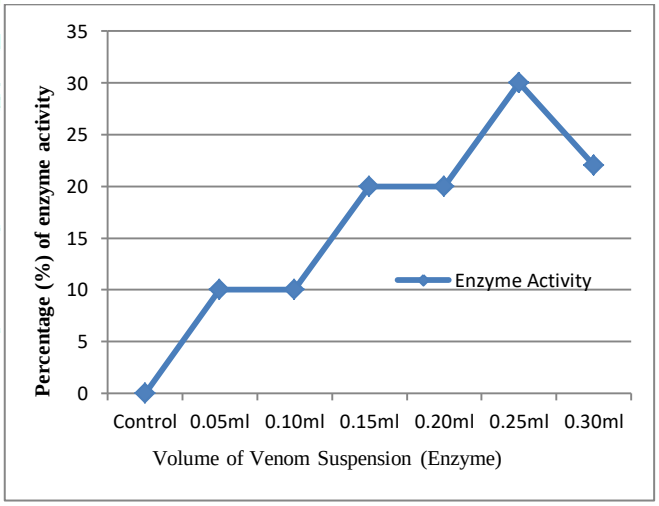

Figure 6: Efficacy of Acetylcholine esterase activity

In inhibition study, the 0.25ml of snake venom was preincubated for 30 minutes at 37˚C with six different concentrations (50µg to 300µg) of acetone extract of *Justicia adhatoda* leaves. In inhibition study, the AEJA was significantly ($p<0.005$) decreased the enzyme activity by dose dependant manner up to 250µg concentration when compared to the venom served alone. The maximum enzyme inhibition activity was observed at 250µg concentration of acetone extract of *Justicia adhatoda* leaves. This result was clearly demonstrates that the AEJA was significantly ($p<0.005$) inhibited the acetylcholine esterase enzyme activity (figure 6a). The observed result was statistically significant ($p<0.005$) when compared to the control

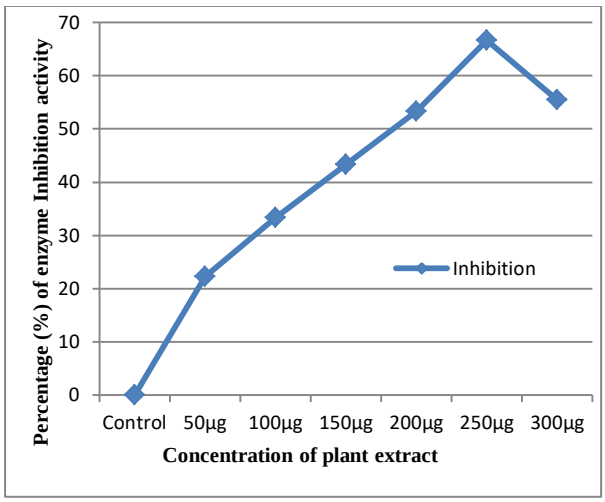

Figure 6a: Inhibition of Acetycholine esterase enzyme activity

4. DISCUSSION

The snake venom is a complex mixture of toxic enzymes, procoagulants, non-enzyme proteins, peptides, carbohydrates, amines, lipids and metal ions. The venom exerts toxic like neurotoxic, cytotoxic and hemotoxic effects. The administration of antisnake venom (ASV) is the only specific treatment for snake bite with many drawbacks. Monovalent ASV is preferable to the polyvalent type since it is less hazardous to the patient and likely to be more effective in the treatment of the particular bite; however, a species diagnosis must be made before the right treatment can be chosen. Polyvalent antivenom carries a risk of severe adverse reactions, and other problems such as difficulty to manage and usage, variety of dosage, and high cost. Furthermore, antivenom sometimes does not provide enough protection against snake envenomation, especially local poisoning.

Many Indian herbal plants have been used for the treatment of snake bites. An ethnobotanical survey of folk plants used in snake bites in southern parts of Tamil Nadu reports the use of 72 medicinal plants in snake bites. Plant extracts of *Aristolochia indica* (terpenoids), *Hemidesmus indica* (phenols), *Gloriosa superba* (esters), *Strychnos nux-vomica*, *Rauwolfia serpentina* (alkaloids), *Eclipta prostrate* (wedelolactone), *Achyranthes aspera* (glycosides) and *Andrographis paniculata* (terpenoids) have shown potent venom neutralizing effect [15].

Neutralization of Russell's viper venom enzymes namely phospholipase, protease and hyaluronidase is reported with the bark extract of *Anacardium occidentale* in a dose dependent manner [16]. *Tamarindus indica* has showed potent venom neutralizing properties. Main hydrolytic enzymes responsible for the early effects of envenomation by Russell's viper (inflammation, local tissue damage, and hypotension) have been inhibited by the seed extract, in a dose-dependent manner [17].

In the present study, the acetone extract of *Justicia adhatoda* leaves was tested against the six different enzymes like 5'nucleotidase, phosphodiesterase, phosphomonoesterase, hyaluronidase, acetylcholine esterase and L-amino acid oxidase that present in Russell's viper venom which is responsible for the lethality. The acetone extract showed a potent enzyme inhibitory effects, the AEJA was showed a significant ($p<0.005$) snake venom inhibition activity. It may due to the presence of various phytochemicals present in the *Justicia adhatoda* leaves. The efficacy of the snake venom enzymes was previously assayed by administering them in alone. The observed results showed the decreased levels of substrate. When the venom concentration was increased the substrate level was significantly ($p<0.005$) decreased. It indicates the activeness of the enzymes present in Russell's viper venom.

Then the venom was tested with six different concentrations i.e., 50µg to 300µg of AEJA. The snake venom was preincubated with the different concentrations of plant extract. The enzyme inhibitory activity was observed in dose dependant manner. When the extract concentration was increased the activity of the enzyme was significantly ($p<0.005$) decreased. The 250µg concentration of acetone extract showed the maximum inhibitory activity in all tested enzymes (Figure 1a-6a).

The 5' nucleotidase is involved in hemostatic alterations by inducing anticoagulant effect by interacting with factors of intrinsic pathway and also by inhibiting platelet aggregation by liberation of adenosine with the help of adenosine diphosphohydrolase degrading adenosine diphosphate to adenosine monophosphate [18]. Dhananjaya *et al.* reported that aqueous extract of stem bark of mangifera indica showed the maximum potent and inhibited phospholipase, protease, hyaluronidase, 5' nucleotidase, ATPase and phosphomonoesterase enzyme present in *Daboia ruselii* snake venom [18]. This result attributed the support to the findings of the present research work. Ushanandini *et al.* reported that dried seed ethanolic extract of *Tamarindus indica* showed the inhibition of phospholipase A2, protease, hyaluronidase, L-amino acid oxidase and 5' nucleotidase enzyme present in Russell's viper snake venom [16]. This result attributed the support to the findings of the present research work.

The phosphomonoesterase enzyme is known to non specifically hydrolyze the ribose and deoxyribonucleotides. They are mostly mettalloenzymes as their activities been inhibited by metal ion chelators [18]. Shirwaiker *et al.* reported that ethanolic extract of *Acalypha indica* to inhibit the venom induced lethality, hemorrhage, cardiotoxic and neurotoxic effects [19]. This result attributed the support to the findings of the present research work. Bhavya *et al.* reported that ethyl acetate extract of *Azima tetracantha* was able to antagonize the activity exhibited by the acetylcholine esterase enzyme present in the viper venom [20]. The inhibition of enzyme activities by the extract may reduce the action of venom or its toxins in the prey or victim.

5. CONCLUSION

In the present study an attempt has been made to present a comprehensive account of antisnake venom activity of *Justicia adhatoda* leaves for the treatment of snake bite. The results clearly showed that the plant *Justicia adhatoda* leaves were showed an effective antisnake venom activity against Russell's viper venom. An ethnic group from herbal remedies against envenomation without antivenom administration and it is the accepted therapy. Plant extracts represent an extremely rich source of pharmacologically active compounds and possess more than one biochemical/pharmacological property. Interaction of such compounds with the toxins/enzymes leads to the neutralization/inhibition of their activities. So plant remedies may be beneficial for the treatment of snakebite and may find alternative to antivenom serum.

6. REFERENCES

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