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Efficacy of arecoline hydrobromide against cattle tick *Rhipicephalus (Boophilus) microplus*

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ABSTRACT

The objective of the present study was to determine the efficacy of arecoline hydrobromide against Rhipicephalus (Boophilus) microplus. Larval packet test (LPT) was performed on larvae produced from collected ticks. Adult immersion test (AIT) was performed to determine live tick weight, the mortality of ticks, and inhibition of oviposition and hatching after arecoline application. Arecoline was taken in different concentrations (i.e. 0.1, 0.5, 1.5, 2.5 5, 7.5, 10 and 12.5 mg/mL) to evaluate its acaricidal activity. The result of LPT exhibited 100% mortality of larvae at 12.5 mg/mL concentration of arecoline. AIT results showed that arecoline caused 87.90% mortality in its highest dose, reduction of egg weight (0.031 g) and excessively reduced hatch rates (2.03%) in ticks as compared to the control group. The level of protective enzymes (SOD and GST) was found to decrease when a reduction in the activity of MAO and AChE enzymes were achieved. It can be concluded from the study that arecoline possess significant acaricidal and oxidative stress-inducing activities in the cattle tick R. microplus. It can be developed as an herbal acaricide in place of synthetic drugs that harm living beings and the environment.

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Areca catechu; larval packet test; adult immersion test; acetylcholine; oxidative stress

1. Introduction

Ticks are highly specilized obligate haematophagous ectoparasites found in mammals, birds, and reptiles, worldwide (Cicculli et al. 2019). Tick infestation has become a major constraint for milk processing industries in India, resulting in a significant effect on the Indian economy (Jain et al. 2020). Ticks have enormous medical and veterinary relevance owing to the direct damage to their hosts and as vectors of a large variety of human and animal pathogens (Nicholson et al. 2019). In recent days, most of the emerging infectious diseases (babesiosis, theileriosis, and many others) that arise from zoonotic pathogens are transmitted by tick vectors. In India, more than 100 tick species have been identified in which *Rhipicephalus* (Boophilus) *microplus* is the most common and widely spread species among cattle and wild animals (Chigure et al. 2017). *Rhipicephalus microplus* is a tropical cattle tick infesting dairy animals (Godara et al. 2015). It is commonly found in the shelter of cattle and reproduces rapidly. It causes reduction of weight and milk production in cattle, causes and several organ injuries (Kang et al. 2013), and transmits various disease causing pathogens such as *Babesia bovis* and *B. bigemina* (Ram et al. 2004; Bhat et al. 2017). It was reported that *R. microplus* can ingest 100 times more blood than its body weight (Gaudêncio et al. 2016).

The biochemical enzymatic activity is one of the major reason of resistance development in ticks (Chigure et al. 2017). It is known that reactive oxygen species (ROS) play an important role in the control of pathogens attack and/or any foreign material entry in ticks and insects (Kalil et al. 2017). ROS has major involvement in several physiological processes such as control of inflammation and injury, protection of cells, differentiation, signalling, and apoptosis (Fang 2004). Ticks protect themselves from acaricidal agents through detoxifying mechanisms including glutathione-S-transferase, esterases, and monooxygenase pathway. It was reported that *R. microplus* possess phagocytic activity along with the generation of ROS due to antioxidant enzymes such as catalase and superoxide dismutase that remove hydrogen peroxide and superoxide, respectively (Pereira et al. 2001). Other mechanisms of protection maybe by decreasing the penetration of acaricidal agents into ticks body, detoxification by ATP binding cassette transporters, behavioural modification, or alteration of the molecular target. Ticks usually generate resistance against the frequent use of chemical acaricides, which leads to severe effects on cattle health and also causes economic loss.

Medicinal plants have been used for treating several diseases and the most effective insecticides and acaricides have been produced using phytoconstituents. The problem of resistance with the frequent use of chemical acaricidal agents is very common. Additionally, the residues of synthetic products in animals milk also reduces the market value of cattle products. Therefore, there is great interest in natural herbal products. Areca nut is a heavily used substance in Asian countries generally consumed with tobacco, ethanol, and caffeine obtained from *Areca catechu* palm. *Areca catechu* ethanol extract has shown insecticidal activity against house dust mite (Jung 2014). Zaman et al. (2017), reported acaricidal activity in *A. catechu* against ticks livestock infesting. The phytoconstituents found in the nut are alkaloids, proteins, fibre, fats, and other mineral matter. The alkaloids found in *A. catechu* are arecoline, arecolidine, isoguvacine, arecaine, guvacoline, arecaidine and coniine (Venkatesh et al. 2018).

Arecoline, a cholinergic agonist is an alkaloidal drug obtained from *A. catechu* present in the saliva of areca nut chewers (Boucher and Mannan 2002; Guh et al. 2006). It has been known for its potential cognition-enhancing effects in Alzheimer patients. It is noticeable that arecoline crosses the blood-brain barrier with a brain/plasma concentration ratio close to unity (Soncrant et al. 1989). It has been reported that arecoline is effective against Fasciola hepatica, cysticercus, and tapeworms (Shakya and Siddique 2018). Thus, based on the acaricidal efficacy of *A. catechu*, the active constituent (Arecoline) was selected to determine the acaricidal activity against larval and adult stages of *R. microplus*.

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2. Materials and methods

2.1. Drugs and chemicals

Arecoline hydrobromide was purchased from Cayman Chemicals, USA. Deltamethrin, Tween 80, chlorodinitrobenzene, pyrogallol, dithiothreitol, phenylmethylsulfonyl fluoride, and ethylenediaminetetraacetic acid were procured from Sigma Aldrich, USA. Leupeptin, chymostatin, soybean trypsin, pepstatin, kynuramine hydrobromide, pargyline HCl and acetylthiocholine iodide were purchased from HiMedia Pvt Ltd. USA. Other chemicals and solvents were of analytical grade and distilled water was used throughout the study.

2.2. Collection, identification and feeding of ticks

Ticks were collected from cattle sheds located at Gokul Nagar, Bhilai (21.1569° N, 81.2656° E). Wide mouth containers were used to collect ticks and then the mouth was covered with muslin cloth. The ticks were identified under a stereomicroscope according to methods given by Walker et al. (2003). The engorged female ticks were collected carefully and allowed to incubate at temperature 27 ± 2°C and 80 ± 5% relative humidity in an incubator in the laboratory. The feeding of ticks was performed according to the method of Trentelman et al. (2017). Ticks and larvae were fed on bovine blood (pre-warmed at 37°C) given through goldbeater's skin as a feeding membrane.

2.3. In vitro acaricidal activity

2.3.1. Adult immersion test (AIT)

AIT was conducted according to the method of Drummond et al. (1976) with little modification. Ticks used in the adult immersion test (AIT) within 24 h of collection and incubated until laying eggs. A total of 100 adult ticks were weighed and assigned to different groups randomly, each group containing ten ticks. The collected ticks were dipped in different concentrations (0.1, 0.5, 1.5, 2.5 5, 7.5, 10 and 12.5 mg/mL) of arecoline hydrobromide prepared in phosphate buffer saline (PBS, pH 7.2) for 2 min. Deltamethrin (0.5%) was used as positive control and distilled water with 2% Tween 80 was used as a negative control. The ticks were allowed to recover from solution and dried by placing on a filter paper in separate Petri dishes. The ticks that were unable to oviposit 14 days and those that produced non-viable eggs considered as dead. LC50 value of arecoline hydrobromide was determined using probit analysis by taking concentration in which tick mortality was initiated to the maximum mortality. The percentage of mortality was determined by using the following formula:

$$\text{Mortality}(\%) = \text{Number of dead ticks}/\text{Total number of ticks} \times 100$$

2.3.2. Larval packet test (LPT)

LPT was conducted by impregnated filter paper with different concentrations of arecoline hydrobromide (0.1, 0.5, 1.5, 2.5 5, 7.5, 10 and 12.5 mg/mL). 14 day old larvae in 100 numbers (approximately) were dropped into impregnated filter packets and sealed on the sides using clamps (Stone and Haydook 1962). The packets were then incubated at a temperature of 27 ± 2°C and relative humidity of 80 ± 5% and subsequent mortality of larvae was quantified after 24 h. The mortality rate was determined according to the following formula:

$$\text{Mortality}(\%) = \text{Number of dead larvae}/\text{Total number of larvae} \times 100$$

2.4. Biochemical enzymatic analysis

Biochemical enzymatic activity was determined by taking 100 ticks per plates containing 5 mg/mL, 10 mg/mL and 12.5 mg/mL of arecoline hydrobromide. The plates were incubated at 27°C with a relative humidity of 80%. Ticks were homogenized in saline (0.3 mL) over ice bath after treatment in every 4 h up to 24 h. It was centrifuged at 3000 rpm for 7 min at 4°C and then the supernatant was collected for enzymatic analysis (Hu et al. 2015).

2.4.1. Superoxide dismutase (SOD) activity

The SOD enzymatic activity was determined by preparing the reaction mixture containing potassium phosphate buffer (5 mmol/L, pH 7) in the volume of 99 µL and 30 µL of ticks homogenate. Pyrogallol (100 µmol/L) was added in the reaction mixture in the volume of 15 µL to start the reaction. The absorbance of the reaction mixture was measured at 540 nm (Oliveira et al. 2018).

2.4.2. Glutathione S-transferase (GST) activity

The GST enzymatic activity was determined by preparing a working solution, containing 2.5 ml reduced glutathione (GSH, 10 mM) in phosphate buffer (pH 6.5) along with the incorporation of chlorodinitrobenzene (63 mM) in the concentration of 125 µl. The working solution (150 µl) along with tick homogenate (10 µl) was added in a microtitre plate (96-well). However, 10 µl water in lieu with tick homogenate was added in the control blank well. These plates were incubated in the dark for 20 min followed by measurement of absorbance at 350 nm (Habig et al. 1974).

2.4.3. Nitric oxide synthase (NOS) activity

NOS activity was measured by observing the conversion of l-arginine to l-citrulline. NOS enzymatic activity was determined by preparing ticks homogenate in a lysis solution containing Tris–HCl (pH 7.4) in the volume of 50 mM, ethylenediaminetetraacetic acid in the volume of 1 mM, sucrose (0.32 M), dithiothreitol (1 mM), NaCl (50 mM) and phenylmethylsulfonyl fluoride (0.2 mM). The inhibitors added in the solution were leupeptin, chymostatin, soybean trypsin inhibitor and pepstatin each containing 10 µg/ml. The centrifugation of homogenates was performed at 15,000 rpm for 30 min and then supernatant passed through a column (Dowex AG 50WX-8) to remove l-arginine (Bredt and Snyder 1990).

2.4.4. Monoamine oxidase (MAO) activity

The monoamine oxidase enzyme activity was determined by preparing a reaction mixture containing tick homogenate (20 µl) in 0.2 M glycine buffer (pH 9.2). It was centrifuged for 3 min at 1000 rpm then the supernatant was collected and stored at cold temperature. The aliquots of homogenate (100 µl) were incubated in a buffer solution containing kynuramine hydrobromide in the concentration of 1.25×10^{-4} M along with inhibitor (pargyline HCl, 1 ml) up to 60 min at 37°C. The addition of 1 M NaOH (100 µl) and 10% $ZnSO_4$ with gentle heating at 100°C for 3 min stopped the reaction. The supernatant was collected after centrifugation in a tube containing 1 M NaOH. The fluorescence of the product was determined using a spectrophotometer at 360 nm (Hall et al. 1982).

2.4.5. Acetylcholinesterase (AChE) activity

For the determination of AChE activity acetylthiocholine iodide (ATChI, 1 mM) was used as a substrate. The tick homogenate (20 µl) was incubated with the substrate at 25°C for 60 min. The measurement of absorbance was performed at 420 nm (Ellman et al. 1961).

2.5. Statistical analysis

Values were shown as mean ± SD. Statistical analysis was performed using One way ANOVA followed by Dunnett's multiple comparison test using Graph Pad Prism software version 8.1. The values were significantly different at *p < 0.05, **p < 0.01 and p***<0.001 in comparison to the control group. Probit analysis was used to determine dose-response data. Regression equation analysis was performed for the probit-transformed data of mortality to determine LPT, AIT and biochemical analysis of arecoline hydrobromide against *R. microplus* (Finney, 1962).

3. Result

3.1. Effect of arecoline in larval packet test

All the concentrations of arecoline hydrobromide were found effective in reducing larvae numbers as shown in Table 1. More specifically, concentration-dependent killing was achieved with the test drug in comparison to control groups. All the concentrations of the drug significantly (***p < 0.001) reduced number of larvae. However, the highest concentration of the drug (12.5 mg/mL) exhibited complete mortality of all larvae (100%). On the other hand, 7.5 mg/mL and 10 mg/mL drug concentrations showed 78.41% and 91.74% mortality. The regression graph of larval probit mortality of *R. microplus* plotted against log values of progressively increasing concentrations of the arecoline is shown in Figure 1. Lethal concentration (LC_{50}) of arecoline for *R. microplus* of the larval packet test (LPT) was 4.16 mg/mL as shown in Table 2.

3.2. Effect of arecoline in adult immersion test

Arecoline hydrobromide was found effective in reducing tick number, egg weight, and hatching capability as shown in Table 3. The percentage mortality was achieved in a dose-dependent manner and the highest mortality was seen with a 12.5 mg/dL dose arecoline (87.90%). The hatching of ticks was reduced to 2.03% with the treatment of arecoline in the highest dose (12.5 mg/mL) in comparison to the control group. The total effectiveness of the product was 82.32%. The egg weight of the ticks was also reduced with the treatment of arecoline hydrobromide. The regression graph of adult ticks probit mortality of *R. microplus* plotted against log values of progressively increasing concentrations of the arecoline hydrobromide is shown in Figure 2. LC_{50} of arecoline hydrobromide for *R. microplus* of the AIT was 2.49 mg/mL as shown in Table 2.

Table 1. Effect of arecoline on larvae in larval packet test.

Groups	Dose (mg/mL)	Mortality (%)
Negative control	-	2.20 ± 2.01
Positive control	0.5	100 ± 0.00***
AH1	0.1	9.55 ± 2.01
AH2	0.5	19.57 ± 1.98*
AH3	1.5	32.54 ± 2.64***
AH4	2.5	49.98 ± 3.02***
AH5	5	62.12 ± 2.94***
AH6	7.5	78.41 ± 2.32***
AH7	10	91.74 ± 2.29***
AH8	12.5	100.00 ± 0.00***

Data are represented as mean ± SEM (n = 100); significantly different at *p < 0.05, **p < 0.01 and ***p < 0.001 in comparison to control group. Negative control – water with 2% Tween 80, Positive control – Deltamethrin, AH – Arecoline hydrobromide.

Table 2. Lethal concentration (LC50) of arecoline for *R. microplus* of the adult immersion test (AIT) and larval packet test (LPT) by probit analysis.

Test	Slope ± SE (95% CL)	R^2 (%)	LC_{50} (95% CL)
LPT	4.73 ± 0.31 (3.96–5.50)	94.39	4.16
AIT	4.63 ± 0.07 (4.43–4.82)	83.26	2.49

3.3. Biochemical estimation

3.3.1. Superoxide dismutase (SOD) level

Figure 3 shows the level of SOD in ticks after treatment with arecoline. SOD level was reduced significantly in treated groups compared to control after 24 h of treatment. Arecoline significantly (***P < 0.001) reduced the SOD enzymatic level in the concentration of 12.5 and 10 mg/mL to 7.50 and 12.30 U/mg protein in comparison to the control group (18.32 U/mg protein). However, it was less significant (*P < 0.05) with a 5 mg/mL dose of arecoline (16.60 U/mg protein). On the other, reduction in the SOD level was lowest with the standard group, Deltamethrin (6.29 U/mg protein).

3.3.2. Glutathione S-transferase (GST) level

GST level in ticks after treatment with arecoline is shown in Figure 4. The level of GST was reduced significantly with all the treated groups in comparison to the control group. The reduction in the level of GST enzymatic activity was more significant (***P < 0.001) with the 12.5 and 10 mg/mL doses of arecoline (41.35 and 62.54 U/mg protein, respectively) in comparison to the control group (83.50 U/mg protein).

3.3.3. Nitric oxide synthase (NOS) level

Figure 5 shows the level of NOS in different treatment groups. NOS activity was significantly (***p < 0.001) raised with the treatment of arecoline at 8.44 and 6.65 U/mg protein in the concentrations of 12.5 and 10 mg/dL, respectively as compared to the control group (3.69 U/mg protein). However, the level of NOS was raised insignificantly with the lowest dose of arecoline to 3.96 U/mg protein. The level of NOS in the Deltamethrin treated group was 7.65 U/mg protein.

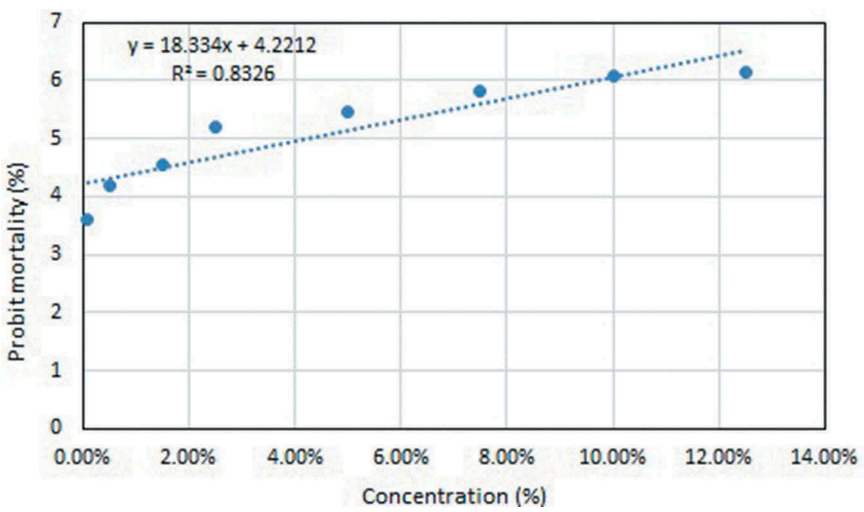

Figure 1. Probit mortality in fully engorged adult *R. microplus* subjected to dose-response AIT assay with arecoline hydrobromide.

Table 3. Effect of arecoline on engorged female ticks in adult immersion test.

Groups	Dose (mg/mL)	Mortality (%)	Total number of eggs	Egg weight (g)	Hatchability (%)	Effectiveness of the product (%)
Negative control	-	0.98 ± 0.23	96.31 ± 2.02	0.071 ± 0.010	76.37 ± 3.17	0.00 ± 0.00
Positive control	0.5	100 ± 0.00***	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100 ± 0.00
AH1	0.1	8.12 ± 2.02***	77.21 ± 2.92	0.062 ± 0.0010	51.01 ± 1.11	3.21 ± 0.32
AH2	0.5	21.77 ± 3.11***	59.54 ± 2.29	0.064 ± 0.009	41.23 ± 2.11	12.32 ± 1.22
AH3	1.5	33.54 ± 2.65***	43.32 ± 2.31	0.061 ± 0.010	35.78 ± 2.15	26.11 ± 2.03
AH4	2.5	58.69 ± 2.94***	34.23 ± 2.43	0.063 ± 0.010	19.27 ± 2.46	33.56 ± 2.77
AH5	5	68.65 ± 2.41***	21.65 ± 2.58	0.059 ± 0.011	11.93 ± 1.87	47.32 ± 2.22
AH6	7.5	79.58 ± 2.83***	13.07 ± 2.32	0.055 ± 0.010	6.43 ± 1.24	61.32 ± 2.26
AH7	10	86.93 ± 3.24***	9.21 ± 2.00	0.034 ± 0.00	2.21 ± 0.23	79.33 ± 2.42
AH8	12.5	87.90 ± 2.93***	9.84 ± 2.39	0.031 ± 0.00	2.03 ± 0.21	82.32 ± 2.65

Data are represented as mean ± SEM (n = 10); significantly different at *p < 0.05, **p < 0.01 and ***p < 0.001 in comparison to control group. Negative control – water with 2% Tween 80, Positive control – Deltamethrin, AH – Arecoline hydrobromide.

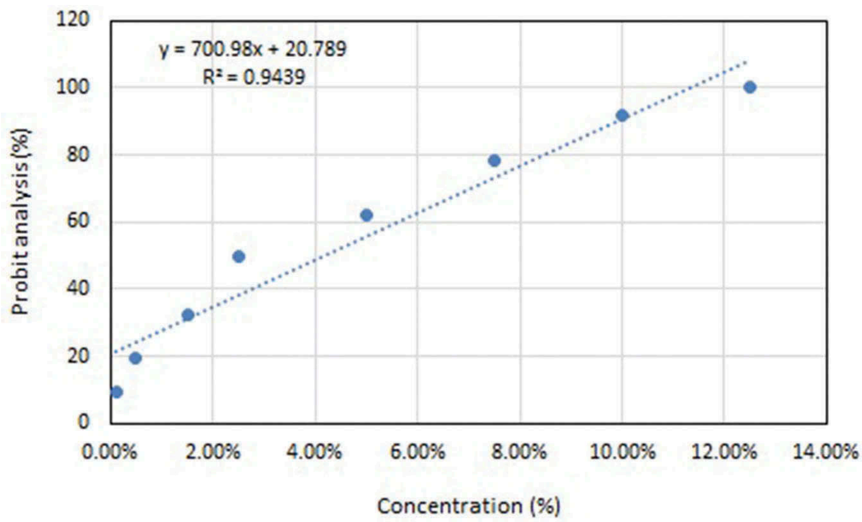

Figure 2. Probit mortality in larvae of *R. microplus* subjected to dose-response LPT assay with arecoline hydrobromide.

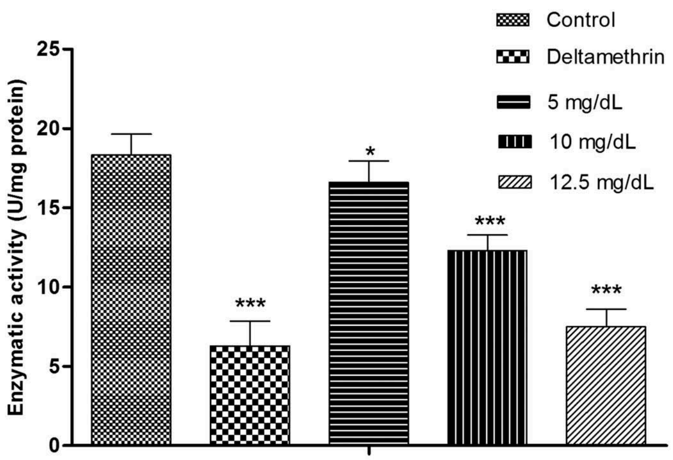

Figure 3. Effect of arecoline hydrobromide on SOD level of ticks Data are represented as mean±SD (n = 10), significantly different at *p < 0.05, **p < 0.01 and ***p < 0.001 in comparison to control group.

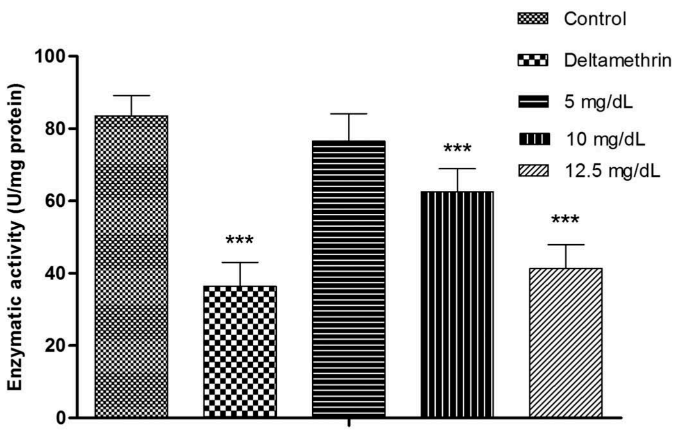

Figure 4. Effect of arecoline hydrobromide on GST level of ticks Data are represented as mean±SD (n = 10), significantly different at *p < 0.05, **p < 0.01 and ***p < 0.001 in comparison to control group.

3.3.4. Monoamine oxidase (MAO) level

The level of MAO was decreased significantly (***p < 0.001) with the treatment of arecoline as shown in Figure 6. The level of MAO was 5.16 and 8.44 U/mg protein with 12.5 and 10 mg/mL doses of arecoline, respectively in comparison to the control group. It was less significant (**p < 0.01) with 5 mg/mL dose (15.35 U/mg protein). The standard group significantly reduced the level of MAO to 3.35 U/mg protein.

3.3.5. Acetylcholinesterase (AChE) level

The change in AChE level after treatment with arecoline is shown in Figure 7. AChE level was reduced significantly (***p < 0.001) with the treatment of arecoline in comparison to the control group. The inhibition of AChE activity after 24 h was 0.26 U/mg protein by 12.5 mg/mL group, 0.40 U/mg protein by 10 mg/mL and 0.52 U/mg protein by 5 mg/mL treated groups in comparison to control group (0.57 U/mg protein). The AChE inhibitory activity by arecoline was found to be concentration-dependent. The

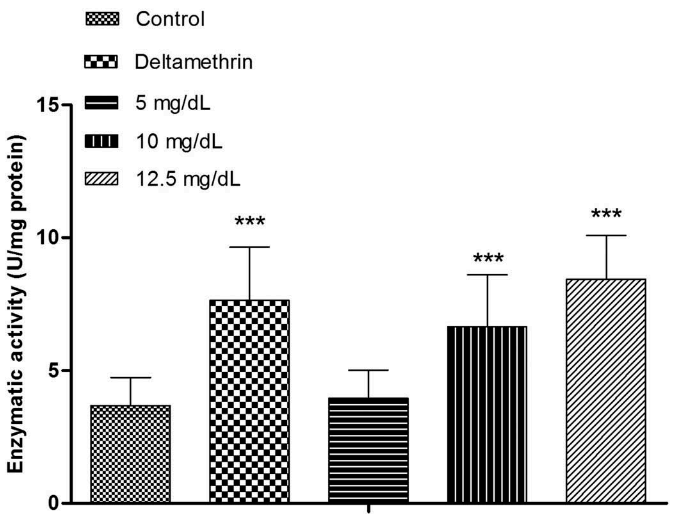

Figure 5. Effect of arecoline hydrobromide on NOS level of ticks Data are represented as mean±SD (n = 10), significantly different at *p < 0.05, **p < 0.01 and ***p < 0.001 in comparison to control group.

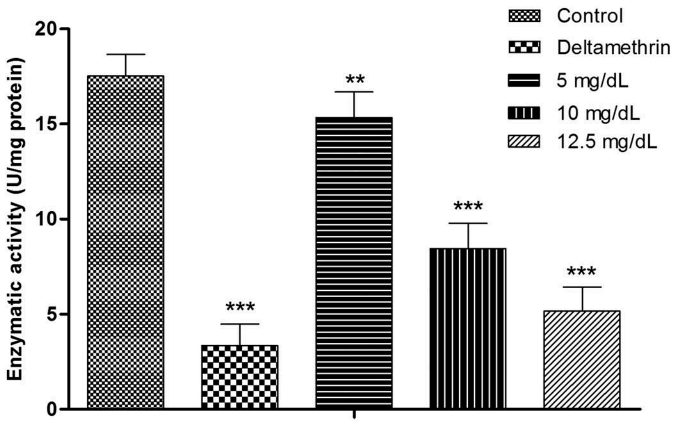

Figure 6. Effect of arecoline hydrobromide on MAO level of ticks. Data are represented as mean±SD (n = 10), significantly different at *p < 0.05, **p < 0.01 and ***p < 0.001 in comparison to control group.

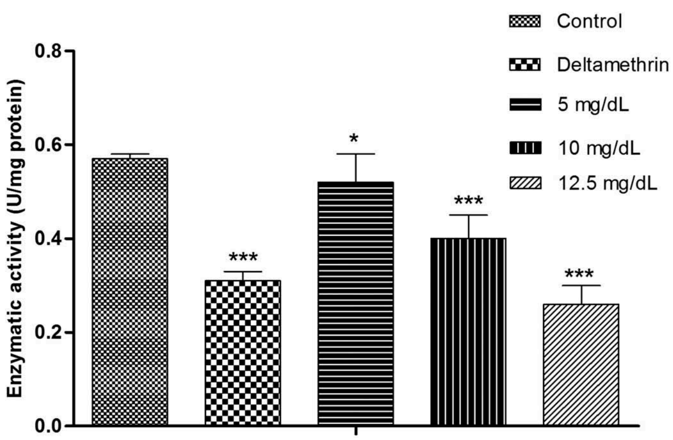

Figure 7. Effect of arecoline hydrobromide on AChE level of ticks. Data are represented as mean±SD (n = 10), significantly different at *p < 0.05, **p < 0.01 and ***p < 0.001 in comparison to control group.

inhibition of AChE activity by Deltamethrin was 0.31 U/mg protein as compared to the control group.

4. Discussion

In the present study, arecoline hydrobromide was used for acaricidal activity. It was found effective in killing ticks and larvae and for reducing egg weight. Jung (2014) reported that *A. catechu* extract was effective against house dust mite due to the presence of arecoline. Other researchers found two active metabolites of arecoline (arecaidine and arecoline N-oxide) that had an impact on the pharmacokinetics of the body. Another study reported that arecaidine produced protein toxicity and arecoline N-oxide showed mutagenicity (Pan et al. 2018). Our results showed that the egg weight of ticks was significantly decreased with 10 and 12.5 mg/mL concentrations of arecoline. Similar results were achieved by Dantas et al. (2015), using leaves of *Neoglaziovia variegata* against *R. microplus*. Results showed that dose-dependent acaricidal activity of arecoline was achieved against *R. microplus*. Our results were as per the work of Gazim et al. (2011), in which 100% mortality of ticks and significant acaricidal activity of *Tetradenia riparia* oil was achieved in the concentration of 12.5%. Other studies of the herbal drug reported similar mortality in the concentration range of 12.5 to 50% (Chagas et al. 2002; Martins 2006). A sub-chronic toxicity study on rats reported that arecoline is toxic only in higher doses and if consumed for a long period. Arecoline prolongs the oestrous cycle with enhanced irregularity in oestrous cyclicity (Kim et al. 2018). The consumption of *A. catechu* cause decreased birth weight and delivery before maturity (Liu et al. 2011). Larval packet tests exhibited promising results. Larvae were completely killed (100%) in 12.5 mg/mL concentration. Even at the lowest concentration (5 mg/mL), the mortality rate was more than 60%. Similar, results were obtained in the study of Politi et al. (2012). A routine screening performed by Bigg and Purvis (1976) and a report published in Nature indicated that arecoline possesses acaricidal activity against *R. microplus*. However, no rationale was given in the published report. The present study was conducted to determine the effect of arecoline in both larvae and adult ticks of *R. microplus* along with its efficacy in the reduction of protective enzymatic levels in ticks.

Arecoline is a main alkaloidal compound obtained from *A. catechu*, it possesses a significant neurotoxic property by enhancing free radical activity in the body (Shih et al. 2010). SOD and GST are the enzymes required for cellular protection against toxic products produced during aerobic respiration. GST catalyzes the reduction of organic hydroperoxides into corresponding alcohols and thus prevents oxidative damage (Allocati et al. 2018). These are the marker enzymes for the increased production of reactive oxygen species (ROS) in the body. ROS mediated cellular damages in the body is shielded by these enzymes (Perry et al. 2010). In our study, arecoline was found destructive drug may be due to promoting the production of excessive O^{2-} radicals in ticks. It caused the reduction of defensive enzymatic level (i.e. SOD and GST) in ticks. Results exhibited that the used acaricide could cause significant oxidative damage in ticks that are associated with marked perturbations in the antioxidant defence system. Results showed that arecoline may reduce the detoxification ability of ticks against toxins; in other words, it could increase its toxicity to ticks.

NOS deals in the biosynthesis of nitric oxide (NO) by converting L-arginine to L-citrulline. nNOS or neuronal nitric oxide synthase is found in the brain and expressed in synaptic terminals of neurons. NO is a free radical that has cytoprotective effects in ticks against toxins (Habib and Ali 2011). In our study, arecoline enhanced the activity of NOS enzyme in ticks; thus, the production of excess NO in nerve tissue leads to excitation of neurons and it might be responsible for the death of ticks. NO is cell-protective at a low level, however, at a higher level, it causes cytotoxicity (Paradise et al. 2010).

MAO is a flavoprotein that exists in the outer mitochondrial membrane of neuronal cells that help in the oxidative deamination of amines (Ramsay 2012; Yildiz et al. 2014). It plays a role in the regulation of several neurotransmitters such as acetylcholine, serotonin, dopamine adrenaline, and other trace amines (Bortolato et al. 2008). The present study exhibited that the application of arecoline caused a reduction of MAO activity in ticks that enhanced the level of neurotransmitter and made ticks overactive and finally dead. It has reported that acetylcholine inhibits MAO-A and B activity in several parts of the rat brain (Osman and Osman 2008). It was reported that *R. microplus* and several insects possess a high level of MAO for their protection. This enzyme is found in excess in the synganglion, salivary glands and peripheral nerves of ticks (Atkinson et al. 1974). They also dictated that tranylcypromine, pargyline and other formamidine acaricides inhibit this enzyme. Pyrethroids and other synthetic acaricides inhibit monoamine oxidase-A that breaks down neurotransmitters (Rao and Rao 1993). Chlordimeform and related compounds also reported inhibiting MAO activity in ticks, mites and lepidopterous insects (Aziz and Knowles 1973).

Acetylcholinesterase (AChE) is an enzyme the interrupts the functioning of the neurotransmitter, acetylcholine (ACh) in the cholinergic pathways of the brain. AChE degrades ACh into choline and acetate and thus, decreases its concentration at the synapse to bind with the receptors. The enzyme inhibition leads to an accumulation of acetylcholine in the brain and hyperstimulation of muscarinic and nicotinic receptors (Colovic et al. 2013). In the present study, arecoline inhibits the activity of the enzyme, acetylcholinesterase and enhances the level of ACh in ticks. A higher concentration of ACh at the synapse leads to intoxication including hyperexcitation, salivation, lacrimation, tremors, convulsions and paralysed muscles that result in death (Hu et al. 2015; Abdelgaleil et al. 2019). In our study, arecoline reduced enzymatic activity of AChE and it might increase the level of ACh at the synapse, a reason in many of the other causes behind the acaricidal activity of arecoline and death of the ticks. Booth (1989) performed a study using catecholamines (adrenergic and dopaminergic drugs) and acetylcholinergic agonists including arecoline against oviposition of *R. microplus*. They found that adrenergic agonists and arecoline including other acetylcholine agonists significantly reduced the egg weight of ticks. However, this study was performed by taking a single dose (25 µg) of test drugs and was unable to define their effects on larvae, hatchability of ticks, mortality concentration and antioxidant system. In our study, the effect of arecoline was evaluated based on all these parameters and it was found effective against *R. microplus*.

Muscle development gets impaired by areca nut due to clustering of acetylcholine receptor at the neuromuscular junction (Chang et al. 2013). It has been reported that arecoline competitively inhibits enzymatic acetylcholine degradation activity of acetylcholinesterase in the nervous tissues (Jaiswal et al. 2008), more specifically by binding to M_1 muscarinic receptor (Ghelardini et al. 2001). This effect is due to its cholinergic agonist activity, which also has a reported cognition enhancement property. In our study, arecoline caused the mortality of ticks, likely due to its cholinergic agonist activity that causes increased Na^+ conduction between the neurons and thus paralysed peripheral part of the ticks due to extra neuronal discharge at the synapse or production of ROS in cortical neurons.

5. Conclusion

Arecoline was found effective in killing ticks and their larvae. It can be a better alternative to chemically and synthetically produced drugs for acaricidal activity. It is an isolated herbal drug that may reduce the chances of side effects to the living beings and environment. Further, studies to determine the exact mechanism of action and developing acaricidal formulations to determine its biological activity on animals are in continuation.

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Disclosure statement

The authors declared no conflict of interest.

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