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Evaluation of the insecticidal action of polyphenolic compounds from *Streblus asper* (Lour.) on the red cotton bug, *Dysdercus cingulatus* (Fab.) (Hemiptera, Pyrrhocoridae)

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ABSTRACT: The study aims to evaluate oxidative stress and the activity of acetylcholinesterase (AChE), upon applying polyphenolic bioinsecticide isolated from *Streblus asper* (PBSA) at a concentration of 0.595 µg/insect (LD_{50}) by topical application on *Dysdercus cingulatus* Fabricius (Red cotton bug- Hemiptera, Pyrrhocoridae). The results demonstrated that the active fraction exhibited significant inhibition in activities of AChE, antioxidant enzymes and Glutathione-S-transferase (GST) and a significant increase in the lipid peroxides (MDA/ TBARS) which led to the fact that *D. cingulatus* became more susceptible to the tested PBSA. The study has provided basic information on the mechanism of action of PBSA that will be promising to develop effective alternatives to synthetic insecticides.

KEYWORDS: Bioinsecticide, mechanism of action, acetylcholinesterase, antioxidant enzymes, lipid peroxides

INTRODUCTION

Cotton (*Gossipium hirsutum* L.) plant is a shrub and is widely cultivated of great economic importance in over 90 countries for its natural fiber and secondly for seeds (Chaudhry, 2010). The red cotton bug, *Dysdercus cingulatus* F. (Heteroptera, Pyrrhocoridae) is an important pest of cotton. Although synthetic chemical insecticides can control it, the side effects are enormous (Vennila *et al*., 2000). Pollution of the environment by pesticides has been increasing due to their use to manage various pests. Bioinsecticides are highly effective, safe, and ecologically acceptable (Arya *et al*., 2022). Recent emphasis is on the use of natural pesticides, which are usually of plant origin. Unlike synthetic chemical pesticides, which leave harmful residues in the aquatic environment (Aktar *et al*., 2009; Mahmood *et al*., 2016) bioinsecticides are biodegradable, specific in action (harmless to non-target organisms), and also possess the ability to counter pest resistance issues caused by synthetic pesticides (Mishra *et al*., 2020).

Streblus asper Lour (Family: Moraceae) is a small tree which is indigenous to tropical countries such as India, Sri Lanka, Malaysia, the Philippines and Thailand (Glasby, 1991). It is a well-known ethnomedicinal plant which is also used in Ayurveda (Singh and Singh, 1987; Singh and Ram, 1988). It finds place in the Ayurvedic Pharmacopoeia of India and an up-to-date and comprehensive review of

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S. asper that covers its traditional and folk medicinal uses, phytochemistry and pharmacology (Rastogi *et al*., 2006). Preliminary study has reported that polyphenolic extracts from the stem bark of S. *asper* possess insecticidal activity against the fifth instar of *D. cingulatus* (Hashim and Devi, 2003) and its partially purified polyphenolic bioinsecticide from *S. asper* (PBSA) had significant effects in the mortality of newly emerged fifth instar *D. cingulatus* (Anila and Hashim, 2022). A study on the mechanism of insecticidal activity of the most active PBSA in *D. cingulatus* by analyzing oxidative stress and inhibition of acetylcholinesterase activity upon topical application using its LD_{50} concentration was taken up.

MATERIALS AND METHODS

The red cotton bugs, were obtained from the laboratory maintained under controlled conditions (temp 28-30°C, RH 95 ± 2 % and a photoperiod of light 12 h; dark 12 h) by feeding soaked cottonseeds. Newly emerged fifth instar insects were used for the experiments. Each treatment contains three replications and fifteen insects were used for each replication.

The stem bark of the plant, *S. asper* was collected from Nagarcoil Forest (Tamil Nadu, India) and was authentically identified. Polyphenolic compounds were extracted from *S. asper* according to the procedure Hashim and Devi (2003). Two compounds were maximum insecticidal activity. The compound I with maximum insecticidal activity was identified as flavanone compound family (Anila and Hashim, 2022). PBSA was used for evaluation of mechanism of action of insecticidal activity by analyzing oxidative stress and inhibition of AChE activity upon its application on *D. cingulatus*.

Newly emerged fifth instar *D. cingulatus* (15 days after of molting) were used and insects were divided into two groups containing 12 each. Ethanol (40%) was topically applied to group I which served as control and PBSA @ 0.595 µg/insect (LC_{50}) dissolved in ethanol (40%) topically applied to the group II insects which served as test. After 12 and 24 hours exposure the haemolymph (40µl) was collected directly into a polystyrene tube containing few crystals of phenylthiourea by making a tiny incision in the antennae of *D. cingulatus* from each group. Hemocytes were removed from the haemolymph by centrifugation at 10000 rpm for 15 min. Separated haemolymph was used for various analyses. The whole brain was minced separately and homogenized with normal saline and centrifuged for 10 minutes at 3000 rpm and the supernatant was used for various analyses. Homogenates (as described) were performed in pools of 12 insects and every enzyme activity determination was the average of 6 independent pools of 12 insects (Daffre and Faye, 1997).

AchE activity was done by the method of Ellman *et al.* (1961). The activity was expressed as Units per milligram protein where 1 Unit = nanomoles of thiocholine liberated per minute.

Catalase (CAT) activity was measured (Maehly and Chance, 1954). The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The specific activity is expressed in terms of Units/ mg protein where 1 Unit = velocity constant per second.

The measurement of superoxide dismutase (SOD) involves generation of superoxide radical by photoreduction of riboflavin and its detection by nitrite formation from hydroxylamine hydrochloride at 543 nm (Das *et al*., 2000). SOD activity was expressed in Units/mg protein where 1 Unit = enzyme concentration required to inhibit OD at 560 nm of chromogen produced by 50 per cent in 1 minute.

Glutathione-S-transferase (GST) activity was measured spectrophotometrically by the method of Habig *et al*. (1974) using S-2,4-dinitrophenyl glutathione (CDNB) as a substrate. The principle of the method is based on measurement of the conjugation of S-2,4-dinitrophenyl glutathione (CDNB) with reduced glutathione. The formation of adduct of CDNB, S-2,4-dinitrophenyl glutathione was monitored by measuring the net increase in absorbance at 340 nm against the blank. The activity of GST was expressed in terms of l mol/min/mg protein.

The glutathione content (GSH) was determined as described by the improved method of Benke *et al*. (1974). The quantity of reduced glutathione was expressed in mg/ g protein or mg/ dl haemolymph.

Thiobarbituric acid-reacting substances (TBARS) were estimated by the method of Niehaus and Samuelsson (1968).

Protein contents of supernatant were determined after TCA (trichloro acetic acid) precipitation (Lowry, 1951) using bovine serum albumin (BSA) as the standard protein. The protein was measured at 670 nm absorbance in a spectrophotometer. Statistical significance was determined by one way Analysis of Variance (ANOVA) in SPSS 20.0 package. The data given in figures are expressed as mean ± SEM, for n = 6 experiments.

RESULTS AND DISCUSSION

Mechanism of insecticidal action of PBSA on AChE activity:

The control insects without any insecticide exposure exhibited an acetylcholinesterase activity of 28 units/ mg protein in haemolymph. After 12 h of PBSA exposure the enzyme activity significantly reduced to 12.95 units/mg protein and after 24 h of exposure again the activity significantly decreased to 7.89 units/mg protein. Similar pattern of inhibition was observed in enzyme activity in brain of bioinsecticide treated insects. Activity of AChE was significantly inhibited in both haemolymph and brain of red cotton bugs treated with PBSA @0.595 µg/insect (LD_{50}) after 12 and 24 h of topical application when compared to control insects (Table 1). The activity

Table 1. Activity of AChE in haemolymph and brain of *Dysdercus cingulatus* exposed to PBSA

Biochemical parameters studied	After 12 hours				After 24 hours			
	Haemolymph		Brain		Haemolymph		Brain	
	Control	Test	Control	Test	Control	Test	Control	Test
AchE (U^1)	28.16 ± 0.803	12.95[a] ± 0.584	42.36 ± 1.75	27.96[a] ± 1.02	27.53 ± 0.679	7.89[ab] ± 0.5511	42.84 ± 1.27	20.33[ab] ±0.694

[1]Unit = nanomoles of thiocholine liberated per minute per milligram protein; Values expressed as mean ± SEM, for n = 6 experiments. [a]test group is compared to control group at p = 0.05. [b]test group after 12 hours is compared to test group after 24 hours at p = 0.05

Table 2. Activity of antioxidant enzymes in haemolymph and brain of *Dysdercus cingulatus* exposed to PBSA

Biochemical parameters studied	After 12 hours				After 24 hours			
	Haemolymph		Brain		Haemolymph		Brain	
	Control	Test	Control	Test	Control	Test	Control	Test
SOD (U^2)	3.17 ± 0.150	1.85[a] ± 0.072	5.39 ± 0.179	3.17[a] ± 0.117	3.08 ± 0.078	1.16[ab] ± 0.046	5.46 ± 0.194	2.26[ab] ± 0.086
CAT (U^3)	16.58 ± 0.358	8.96[a] ± 0.142	32.47 ± 0.909	25.49[a] ± 0.610	16.44 ± 0.252	6.32[ab] ± 0.157	33.54 ± 0.708	18.67[ab] ± 0.424
GST (U^4)	3.46 ± 0.096	1.84[a] ± 0.045	6.31 ± 0.117	4.25[a] ± 0.080	3.22 ± 0.061	0.924[ab] ± 0.029	6.43 ± 0.104	3.09[ab] ± 0.070

Values expressed as mean ± SEM, for n = 6 experiments; [a]test group is compared to control group at p = 0.05; [b]test group after 12 hours is compared to test group after 24 hours at p = 0.05. [2] Unit = enzyme concentration required to inhibit OD at 560 nm of chromogen produced by 50 % in 1 minute. [3] Unit = velocity constant/ second; [4] Unit = m M of CDNB utilized /min/mg protein

was significantly inhibited after 24 hours of topical application when compared to 12 h exposure.

Activity of catalase and superoxide dismutase **(**SOD) was significantly inhibited in both 12 and 24 h time intervals after the topical application of PBSA @ 0.595 µg/insect (LD_{50}) and the inhibition was more pronounced in 24 h exposure in both haemolymph and brain. There was a significant reduction in GST activity in the haemolymph and brain of insects after the topical application of PBSA at 0.595 µg/insect (LD_{50}) for 12 and 24 h time intervals when compared to control insects (Table 2). The inhibition was more pronounced in the haemolymph and brain after 24 h exposure when compared to insects treated for 12 h.

Duration dependent decrease in GSH content was observed in both haemolymph and brain of PBSA at 0.595 µg/insect (LD_{50}) treated insects when compared to control insects. The decrease was higher in 24 h treated insects when compared to 12 h treated insects. TBARS content was significantly increased in haemolymph and brain after 12 and 24 h exposure of PBSA at 0.595 µg/insect (LD_{50}) treated insects when compared to control group (Table 3). There was significant increase in TBARS level in 24 h treated insects when compared to 12 h.

In the present study the polyphenolic bioinsecticide exposure have been demonstrated to reduce the activity of acetyl cholinesterase significantly in red cotton bugs. The results are in agreement with Maazoun *et al.* (2017) who reported that *Urginea maritima* bulbs extract exhibited inhibitory AChE activity in *Sitophilus oryzae* (L.). Pesticide-induced oxidative stress is the final manifestation of a multi-step pathway, resulting in an imbalance between pro-oxidant and antioxidant defense mechanisms. Concomitantly, pesticide intoxication induces a derangement of certain antioxidant mechanisms in different tissues, including alterations in antioxidant enzymes and the glutathione redox system (Banerjee *et al*., 2001). Therefore the attempt to analyze the antioxidant status and measure the activity of AChE in red cotton bugs exposed to PBSA which belongs to a flavanone family, showed a significant inhibition in the activity of AChE and antioxidant enzymes.

Reactive oxygen species (ROS), such as superoxide anions (O2) and H_2O_2 are produced throughout the cells during normal aerobic metabolism. The intracellular concentration of ROS is a consequence of both their production and their removal by various antioxidants. A major component of antioxidant system in mammalian cells consists of three enzymes, SOD, CAT and (glutathione peroxidase) GPX. These enzymes work in concert to detoxify O_2 and H_2O_2 in cells. It has been established that many pesticides are capable of inducing oxidative stress by overwhelming or modulating cellular drug metabolizing systems (Sule *et al*., 2022).

Oxidative stress occurs when there is an imbalance between free radical generation and antioxidant

Table 3. Concentration of reduced glutathione (GSH) and TBARS content in haemolymph and brain of *Dysdercus cingulatus* exposed to PBSA

Biochemical parameters studied	After 12 hours				After 24 hours			
	Haemolymph		Brain		Haemolymph		Brain	
	Control	Test	Control	Test	Control	Test	Control	Test
MDA (TBARS)	0.925 ± 0.047	1.73[a] ± 0.050	1.42 ± 0.053	2.13[a] ± 0.072	0.930 ± 0.042	2.24[ab] ± 0.075	1.42 ± 0.061	2.55[ab] ± 0.068
GSH	16.58 ± 0.592	9.47[a] ± 0.376	24.63 ± 0.581	12.38[a] ± 0.314	17.16 ± 0.551	6.18[ab] ± 0.330	25.24 ± 0.778	8.59[ab] ± 0.424

Values expressed as mean ± SEM, for n = 6 experiments; [a] test group is compared to control group at p = 0.05; [b] test group after 12 hours is compared to test group after 24 hours at p = 0.05

defenses. It often results in severe pathological consequences, such as membrane disruption, DNA damage and protein damage and cytotoxicity (Saini *et al*., 2023). The activities of the antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST) were decreased by fenitrothion incubation. The same treatment reduced the level of antioxidant glutathione (GSH). The activities of glutathione-S-transferase (GST) and gamma-glutamyl transpeptidase (gamma-GT) were more affected by fenitrothion and endosulfan, respectively, indicating oxidative stress (El-Shenawy, 2010). These support the present findings that the significant decrease in antioxidant enzymes in haemolymph and brain of bioinsecticide-treated insects when compared to control.

Recent reports showed that there were significantly reduced GSH levels in all tissues after methiocarb administration in experimental animals (Ozden *et al*., 2009) and methomyl decreased AChE, superoxide dismutase (SOD) and glutathione S-transferase (GST) activities and increased level of lipid peroxidation (LPO) (Mansour *et al*., 2009). These reports are in support with the present findings that the phenolic compound isolated from *Streblus asper* (PBSA) exhibited the insecticidal activity as evidenced by its inhibitory effect on the activities of AChE and antioxidant enzymes and significant increase in the level of lipid peroxidation.

In conclusion, polyphenolic bioinsecticide from *Streblus asper* (PBSA) has significant effects on newly emerged fifth instar *D. cingulatus*, and they caused increased mortality in a duration-dependent manner. The AChE activity and antioxidant status are impaired after the exposure of PBSA to red cotton bugs. The abnormal change in antioxidant status and acetylcholinesterase activity in cotton bugs may be the reason for the insecticidal action. The compound PBSA may therefore serve as an effective alternative to conventional insecticides in controlling red cotton bugs.

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