Schizophyllum commune induced oxidative stress and immunosuppressive activity in *Spodoptera litura*

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Abstract

Background: In a search for the mechanism of action of an endophytic fungus *Schizophyllum commune* against *Spodoptera litura*, the effect of its ethyl acetate extract was judged on antioxidant and detoxifying enzymes as well as on morphology of haemocytes.

Results: Ethyl acetate extract of *S.commune* was administrated to the larvae of *S.litura* using artificial diet method having concentration 276.54µg/ml (LC_{50} of fungus). The effect on antioxidant and detoxifying enzymes (Catalase, Ascorbate peroxidase, Superoxide dismutase, Glutathione-S-Transferase) was observed using haemolymph and midgut of insect larvae for different time intervals (24, 48, 72 and 96). In additionally, haemocytes morphology was also studied using Scanning Electron Microscopy (SEM) after 96hr. In particular, to resist the toxicity, the activities of the antioxidant and detoxifying enzymes (Catalase, Ascorbate peroxidase, Superoxide dismutase, Glutathione-S-Transferase) significantly ($p \leq 0.05$) increased in both the tissues of treated larvae as compared to control. The exposed group revealed various deformities in haemocytes of *S.litura* like breakage in the cell membrane, cytoplasmic leakage and appearance of strumae etc.

Conclusion: This study provides important information regarding the oxidative stress causing potential and immunosuppressant nature of *S.commune* against *S.litura* and elucidates the patterns of antioxidant and detoxifying enzymes activities and changes in haemocytes in the susceptible larvae.

Background

Endophytes are ubiquitous in nature and often colonized in various parts of host plants without showing visible symptoms (Hartley and Gange 2009; Saikkonen *et al*. 2006). Through the mutualistic relationship with their host plant, they enhance plant's tolerance to biotic and abiotic stresses. Various studies revealed that plants infected with endophytic fungi showed resistance to herbivory. Several fungal species viz. *Cladosporium herbarum*, *Rhodotorula rubra*, *Alternaria alternata*, *Epicoccum nigrum*, *Penicillium* sp., *Fusarium graminearum, Cryptococcus* spp., have been reported to protect plants from herbivores (Larran *et al.* 2001; Larran *et al*. 2002; dosSouza and dosSantos 2017). Some of fungal endophytes act as insect pathogenic agents and reported to infect different insect species including lepidopterous larvae, aphids, thrips, and many other cosmopolitan insects having great concern in agriculture worldwide. They infect specific hosts, attributing little or no threat to non-target organisms or beneficial insects (Akutse *et al*. 2014). The anti-insect properties of endophytes have been documented by various researchers (Daisy *et al*. 2002; Lacava and Azevedo 2014; Nazir and Rahman 2018).

Many insect pathogenic fungi such as *Beauveria bassiana, Clonostachys rosea, Isaria farinosa*, *Fusarium oxysporum, Hypocrea lixii, Gibberella moniliformis*, and *Trichoderma asperellum* have been isolated as naturally occurring endophytes from asymptomatic plant tissues (Bills and Polishook 1991; Cherry et al. 1999; Pimentel et al. 2006; Vega et al. 2008; Orole and Adejumo 2009; Akello 2012; Akello and Sikora 2012; Akutse et al. 2013). The insecticidal activity of fungi can be attributed to different secondary metabolites produced by them. Mycotoxins viz aflatoxins, fumonisins, ochratoxins, zearalenone, have great importance in agriculture for pest management (Huffman *et al.* 2010). Various fungal secondary metabolites like avermectins, pantherine, destruxins, ibotenic acid, and tricholomic acid were found to be highly active against insects (Busi *et al*. 2014).

Numbers of researches have been done on the role of fungal endophytes as insect pathogenic but many of them failed to address the mode of action. In order to discover the insecticidal potential the effect on antioxidant and

detoxifying enzymes in insects should be evaluated. In healthy animals, balance between the production and elimination of reactive oxygen species (ROS) occurs but imbalance between the production and detoxification of ROS by biological system resulted to oxidative stress (Rahman and Adcock 2006). Insects have a complex enzymatic and non-enzymatic defense system to encounter oxidative stress. The system regulates the level of lipid peroxidation and mitigates damage to DNA and proteins and other cytotoxic effects (Felton and Summers 1995). The main antioxidant enzymes in insects are catalase (CAT), Ascorbate peroxidase (APOX) and superoxide dismutase (SOD) (Felton and Summers 1995; Wang *et al*. 2001). SOD catalyzes the dismutation of superoxide radical into oxygen and H_2O_2. CAT and APOX catalyze the dismutation of H_2O_2 into oxygen and water. Another detoxifying enzyme, glutathione-S-transferase (GST), eliminates hydroperoxides from the cells (Ahmad *et al*. 1995; Dubovskiy *et al*. 2008). Various xenobiotics incite the production of reactive oxygen species (ROS) and unbalance the antioxidant–pro-oxidant equilibrium, ultimately induce oxidative damage and increase insects' mortality (Freeman and Crapo 1982; Harris 1992; Adamski *et al*. 2003; Krishnan and Sehnal 2006; Hyrsl *et al*. 2007). Such effects arise either from pro-oxidant activity which can be observed as lipid peroxidation or altered antioxidant enzyme activity. So these parameters are significant while estimating of the stress caused by xenobiotics.

Insect haemocytes play active role in providing immunity. They consists the mixture of cells having different morphological and biological functions. The hemocytes are competence in discriminating stranger agents, mediate phagocytosis, encapsulation, cytotoxicity, wound repair and coagulation (Lanot *et al*. 2001; Lavine and Strand 2002). So they played important role in providing defense against parasites, pathogens and other foreign bodies enter in the hemocoel (Ratcliffe and Gagen 1976; Ratcliffe *et al*. 1985; Lackie 1986; Brookman *et al*. 1989; Hoffmann 1994; Lavine and stand 2002). Several studies revealed the effect of biopesticides on haemocytes count but the morphological alterations in haemocytes have not been studied. As haemocytes play important role in providing cellular immune defense so negative impact on haemocytes also reflects immunosuppressant nature.

On the basis of aforementioned discussion, the study was conducted to decipher the effect of ethyl acetate extract of *Schizophyllum commune* on activity of antioxidant enzymes and morphology of haemocytes of *S.litura*, one of the major polyphagous pest.

Results

The results of present study have been depicted in tables 1-4 and figs 1-3. Statistically significant increase in activities of all enzymes with respect to control in both haemolymph and midgut of the exposed *S.litura* larvae was observed.

Table-1 revealed the significant increase in CAT activity within both haemolymph and the midgut (t test, $p \leq 0.05$) of treated larvae as compared to control. Maximum hike was observed at 96hr where value increased from 14.06±0.90 µmole/ml (control) to 51.87±0.36 µmole/ml (exposure group) in haemolymph ($t=38.91$, $p \leq 0.01$) and 17.18±0.56 µmole/mg (control) to 66.56±1.98 µmole/mg (exposure group) in midgut ($t=24.00$, $p \leq 0.01$). Measured values of CAT in the haemolymph and midgut were 3.68 and 3.87 fold higher than control one, for 96hr exposure time group respectively. The difference between all exposure time groups was statistically significant in both haemolymph and midgut as revealed by ANOVA. Further Tukey's test revealed the significant change in enzyme activity between 24hr, 48hr and 72hr exposure groups in haemolymph ($F=63.04$, $p \leq 0.01$) however in midgut significant changes were observed in the activity of catalase between all exposure groups (Tukey's test) ($F=116.06$, $p \leq 0.01$).

Change in Ascorbate peroxidase activity in haemolymph and midgut due to ingestion of ethyl acetate extract of *S. commune* is given in Table. 2. In the haemolymph the enzyme activity was found to be significantly increased to 81.31±1.36µmole/ml in 24hr treatment group from 24.15±2.72µmole/ml in control group ($t=18.79$, $p\leq0.01$). In midgut, 24hr group showed increase in activity to 109.60±2.72 µmol/mg from 45.07±1.87 µmol/mg in control ($t=19.52$, $p\leq0.01$). Similarly significant increase in the Ascorbate peroxidase activity was found in 48hr, 72hr and 96hr exposure group as compared to control in both haemolymph and midgut. Maximum increase was observed at 96hr where activity increased from 29.46±2.72 µmole/ml (control) to 236.00±2.89 µmole/ml (exposed group) in haemolymph ($t=652.72$, $p\leq0.01$) and 53.03±2.72 µmole/mg (control) to 282.26±5.10 µmole/mg (exposed group) in midgut ($t=38.51$, $p\leq0.01$). The effect of duration was found to be significant (One way ANOVA) in both haemolymph ($F=652.72$, $p\leq0.05$) and midgut ($F=411.58$, $p\leq0.01$). Further significant difference between the enzyme activity was found between all the exposure groups in both haemolymph and midgut (Tukey's test) (Table. 2).

The activity of SOD was also found to be higher in both haemolymph and midgut of exposed larvae (Student's t-test) (Table. 3). Maximum hike was observed in 96hr exposure group where 1.53 fold (haemolymph) and 4.14 fold (midgut) increase was observed as compared to control groups. With increase in time duration the enzyme activity was found to be increased ($F=208.79$, $p\leq0.01$) but significant difference was observed only after 96hr exposure as revealed by Tukey's test in haemolymph. In midgut the duration effect was found to be significant ($F=474.91$, $p\leq0.01$) however significant change was observed between 72hr and 96hr exposure groups (Tukey's test).

The ethyl acetate extract of *S. commune* also affected the GST activity. After treatment with ethyl acetate extract of *S. commune* the GST activity in haemolymph and midgut tissue of *S. litura* was significantly increased in all exposure groups as compared to control. The enzyme activity increased up to 72hr and then slight decrease was observed in 96hr exposure group in both tissues, the values being 21.66±1.20 µmol/ml and 19.79±0.84 µmol/ml in 72hr and 96hr exposure groups respectively in haemolymph. In midgut it was decreased in 96hr exposure group i.e. 30.00±0.72 µmol/mg (72hr exposure group) to 26.46±0.84 µmol/mg (96hr exposure group). The effect of duration was found to be non significant in haemolymph however in midgut with increase in time duration the enzyme activity was increased as revealed by ANOVA ($F=189.03$, $p\leq0.01$) and significant difference was found between all the exposure time groups (Tukey's test) (Table.4).

The scanning electron microscopy studies revealed that the haemocytes of *S.litura* were changed very apparently after treatment with *S.commune* ethyl acetate extract (Fig. 1, 2, 3). After 96 hr various morphological deformities were observed in different types of haemocytes. As compared to normal plasmatocytes treated ones showed cell membrane shrinkage, breakage in membrane and cytoplasmic leakage (Fig.1). Similarly normal granulocytes not shown any deformity but treated ones showed strumae and surface abnormalities (Fig.2). Prohaemocytes showed cell membrane shrinkage after treatment with *S.commune* (Fig.3). Overall SEM studies revealed that morphology of haemocytes was highly disrupted after treatment with ethyl acetate extract of *S. commune* for 96hr which might be leads to cytotoxicity. The percentage of cells showing various deformities are significantly very high in exposed group as compared to control group. Exposed group showed 85.00% deformities as compared to 6.66% in control (Fig.4).

Discussion

Under stress conditions, large quantities of reactive oxygen species (ROS) produced by insects (Ding *et al*. 2015), and these excessive ROS may cause damage to the organism itself (Li *et al*. 2018). There are free radical

scavenging systems in insects, such as various protective enzymes including SOD, CAT, and APOX whichl work co-coordinately to maintain the state of dynamic equilibrium in organism, keeping ROS level low to prevent the cellular damage (Gao *et al*. 1995; Kontogiannatos *et al*. 2011; Zhang *et al*. 2019).

The present findings demonstrate the influence of ethyl acetate extract of an endophytic fungus, *S.commune* on the activities of antioxidant enzymes in *S. litura* larvae at different times. The fungal metabolites were found to significantly alter the activities of enzymes with increasing exposure time. This might be due to a host response initiated after toxicity induced by *S.commune*, in which enzymes activity was remarkably affected to metabolize the ROS, reaching the maximum value at 96hr. Previous studies have shown that after fungal infection, protection systems of insects are activated to ward off infection and maintained the normal physiological activities (Song *et al*. 2002; Zhang *et al*. 2003; Ding *et al*. 2015).

At 24hr of fungal extract treatment the SOD activity increased, suggesting the increase in O2 consumption lead to substantial increase in the free radical HO−. This might activated SOD to clear the excess free radicals from the insect body. At 96hr, the SOD activity reached its maximum level, as compared to control 1.53 fold and 4.14 fold hikes was observed in haemolymph and midgut tissue respectively. CAT and APOX activities were also increased to get over the effects of the toxins. *In vivo* metabolism produced large amount of H_2O_2. This might lead to increase the CAT and APOX activity. CAT activity increased 3.69 fold and 3.87 fold at 96hr as compared to control in haemolymph and midgut of *S. litura* respectively. APOX activity also found to be maximum at 96hr. This finding is in line with findings of Karthi *et al.* (2018) who observed increase in superoxide dismutase (SOD), catalase (CAT), peroxidases (POX) activity under the influence of *A. flavus*.

Various detoxification enzymes in insects can effectively metabolize the exogenous toxic compounds (Zhang *et al*. 2001) and play very important roles in maintaining the normal physiological activities in the body (Kontogiannatos *et al*. 2011). GST as a detoxifying enzyme serves a variety of physiological and metabolic functions (Su *et al*. 2007). The enzyme catalyzes the harmful compounds with glutathione and helps to discharge various potential toxic compounds from the body in a non-enzymatic fashion (Ding 2007). In this study, we found that after 24 hr, GST activity increased significantly to ward off the toxicity induced by extract and maintain the normal physiological functions in the insect. At 72 hr, GST activity reached its maximum level. However, at 96 hr the GST activity declined might be due to the damage caused by fungal extract. This indicated that with progressing exposure time toxicity increase, the larvae were unable to synthesize GST and eventually died. Previously Gholamzadeh-Chitgar *et al*. (2017) indicated elevated activity of GST in response to entomopathogenic fungus *B. bassiana*.

Scarce reports illustrated the effect of fungal agents on protective enzymes activities. Ding *et al*. (2015) reported the alteration in *detoxifying and protective enzymes activities under the influence of B. bassiana* in *Xylotrechus rusticus* (Linnaeus). Similarly infection of *Periplaneta americana* (Linnaeus) and *Locusta migratoria* (Linnaeus) with *M. anisopliae* caused marked alteration in level of antioxidant and detoxifying enzymes activities (Mutyala *et al*. 2013; Narenbabu *et al*. 2013, 2014; Jia *et al*. 2016). The entomopathogenic fungus *Hirsutella thompsonii* was also reported to induced variable trend in antioxidant enzymes activities in *P. americana* (Chaurasia *et al*. 2016). However, alteration in enzymes activities in insects under the influence of different stress factors has been reported by many studies (Hyrsl *et al*. 2007; Aslanturk *et al*. 2011). Barata *et al*. (2005) demonstrated the increase in CAT activity along with intensify lipid peroxidation in larvae of *Hydropsyche exocellata* (Dufour) after exposure to cadmium. Sezer and Ozalp (2015) observed enhanced antioxidant enzymes activity in *Galleria mellonella*

(Linnaeus) due to pyriproxyfen. Similarly the effect of UV radiations on antioxidants defense system has been documented by Karthi *et al*. (2014) and Ali *et al*. (2017).

Cellular immune response in insect immune system acts as an important barrier to the infection process (Hoffmann 1995, 2003). Haemocytes types and their specific responses while insect–pathogen interaction act as a good indicators of insect defense reactions (Da Silva *et al*. 2000; Gillespie *et al*. 2000). There are different classes of haemocytes which have been morphologically and functionally characterized in various insects. (Lavine and Strand 2002; Giulianini *et al*. 2003; Costa *et al*. 2005; Giglio *et al*. 2008). The most common types of haemocytes reported in the literature are prohemocytes, granulocytes, plasmatocytes, and oenocytoids. Multifunctional role of haemocytes are phagocytosis, encapsulation, cell agglutination, detoxification etc. Change in number and configuration was observed in haemocytes under different stresses which ultimately affect the health of insects. Consequently these cells have been used to ascertain the cytogenetic damage by toxic chemicals (Begum and Gohain 1996; Gayfullina *et al*., 2006).

The present findings also demonstrate various morphological deformities observed under scanning electron microscope (SEM) in the haemocytes of *S.litura* due to cytotoxic effect of ethyl acetate extract of *S.commune*. As compared to control various cellular deformities were observed in treated ones like cell perforations, rupturing of haemocytes with cytoplasmic leakage and irregular variation on the surface of haemocytes. The abnormalities observed here in were similar to those of destruxin A which have resulted in cell perforation and rupturing with cytoplasmic leakage in haemocytes of *Bombyx mori* (Linnaeus) (Fan *et al*. 2013). There are very less reports available in literature revealing the abnormalities of insect haemocytes using SEM. This is the first finding reporting the alterations in different types of haemocytes in *S.litura* under SEM. The technique has been used to observe and characterize the haemocytes of different insects by various workers (Silva *et al*. 2002; Falleiros *et al*. 2003) and to observe the spores accumulation in the body of insect after fungal infection (Bawin *et al*. 2016a, b; Baggio *et al* 2016; Duan *et al*. 2017). However, similar type of morphological changes as observed in present finding were demonstrated by other workers due to entomopathogenic fungi and insecticides under light microscopy (Ferrarese *et al*. 2005; Habeeb and Abou El-Hag 2008; Kaur *et al*. 2011; Thakur *et al*. 2014; Kaur *et al*. 2015).

S.commune induces various cellular abnormalities as observed under SEM in a present investigation, which might brings cell death and resulted cytotoxic effect as reported earlier (Kaur *et al*. 2018). Haemocytes document a key role in providing cellular immune defense. Since, any agent has negative impact on haemocytes directly influence on cellular immune defense of insect and act as immunosuppressant. So in addition to oxidative stress causing potential, the study also highlights the immunosuppressant activity of *S.commune* on *S.litura*.

Conclusion

In conclusion *S.commune* altered the antioxidant and cellular defense system of *S.litura* as determined by measuring antioxidant enzymes activity and morphology of haemocytes. The finding helps to identify the insect defenses that could be manipulated to accelerate host death in biological control scenario.

Methods

Rearing of *Spodoptera litura*:

Spodoptera litura (Lepidoptera) eggs were obtained from the cauliflower fields around Amritsar (India). After hatching of eggs larvae were fed on castor leaf. Subsequent generations of culture were maintained in laboratory at 25±2°C temperature, 65±5% relative humidity and 12:12 (D: L) photoperiod.

Fungal culture isolation, production and identification

Endophytic fungus was isolated from leaves of *Aloe vera* collected from Amritsar (India). The leaves were thoroughly washed with distilled water, followed by sterilization with 70% ethanol (2 min), 5% sodium hypochlorite solution (5 min) and finally rinsed with sterile distilled water. Sterilized samples were cut into small pieces. Pieces were placed on water agar plates having ampicillin (200mg/ml) as antibacterial agent and incubated at 30°C. After emergence of hyphae, the hyphae tips were picked and cultured on PDA (potato dextrose agar) plates. The culture was purified and maintained on PDA for further studies (Singh *et al*. 2012).

The production was carried out in 50ml malt extract (malt extract=20g/l, dextrose=20g/l,peptone = 1g/l, pH=5.5) broth in 250ml Erlenmeyer flask by inoculating one plug (1 cm square) taken from the periphery of an actively growing culture. The flasks were incubated at 30ºC and 250rpm for 10 days. After 10 days extraction was carried out twice using ethyl acetate at 120rpm and 40ºC. The extracts were concentrated by using rotavapor and dissolved in 1ml DMSO and stored at 4°C. The fungus was identified as *Schizophyllum commune* on morphological and molecular basis as indicated in our previous study (Kaur et al. 2018) by using ITS1 and ITS4 primer to amplify ITS1-5.8S- rDNA- ITS2 region. Amplified ITS region was Purified and sequenced at first base sequencing (Malaysia). The sequence similarity was matched with other available databases retrieved from NCBI using BLAST (Sharma *et al*. 2008).

Toxicity test:

On the basis of bioassay studies the LC_{50} value of ethyl acetate extract of *S. commune* was found to be 276.542µg/ml (Kaur *et al* 2018). This concentration is selected for to analyze its effect on antioxidant and detoxification enzymes and to decipher various morphological changes in haemocytes .

Antioxidant enzyme activities:

To evaluate the effect of fungal extracts on antioxidant enzymes, the third instar larvae (12 days old) were fed with fungal extracts supplemented diet having concentration 276.54µg/ml. The enzyme activities [Superoxide dismutase (SOD), catalase (CAT), Ascorbate peroxidase (APOX) and Glutathione-S-Transferase (GST)] were analyzed in haemolymph and midgut of third instar (12days) larvae.

Larvae were divided into two groups, treatment and control. Treatment group was exposed with LC_{50} of fungus at controlled temperature 25±2°C and relative humidity 65±5%. The second group was treated with control diet (0.5% DMSO) at same conditions of temperature and relative humidity. The effect of fungal extract has been recorded after different time intervals (24hr, 48hr, 72hr and 96hr) in enzyme activities. The experiment was replicated three times. For each treatment and control there are 10 larvae per replication were taken.

Tissue collection

Haemolymph was collected by cutting proleg with microscissor from 10 different larvae fed with same concentration and then it was pooled. Pooled haemolymph (10%) was mixed with PBS (Phosphate Buffer Saline pH 7.0) containing 0.01%phenylthiourea and centrifuged for 20 min at 10000g, 4ºC and supernatant obtained was

used for enzyme activities studies. Similarly midgut tissue was also taken after dissection with microscissor from 10 different larvae fed with same concentration and homogenate (10% w/v) was prepared by homogenizing larval midguts (100 mg in 1 ml) in PBS. Afterwards homogenate was centrifuged in PBS for 20 min at 10000g, 4ºC and supernatant obtained was used for enzyme activities studies.

The extraction procedure was same for all enzymes.

Catalase (CAT) activity:

Enzyme activity was estimated according to methodology given by Aebi (1984) with slight modifications. 0.1ml of supernatant was added into 2.9ml of H_2O_2 in a cuvette. Decrease in absorbance was read at 240nm for 5min at 1min interval (25□C). The enzyme activity was expressed as µM/ml (haemolymph) and µM/mg (midgut) weight.

Ascorbate peroxidase (APOX) activity:

The enzyme activity was calculated according to methodology given by Asada (1984) with slight modifications. 0.1ml of sample, 0.6ml extraction buffer (50mM potassium phosphate buffer pH 7.0) and 0.125ml of 0.3%H_2O_2 were taken in cuvette. The decrease in absorbance was recorded at 290nm for 5min at 30sec interval (25□C). The enzyme activity was expressed as µM/ml (haemolymph) and µM/mg (midgut) weight.

Superoxide dismutase (SOD) activity:

The enzyme activity was calculated according to methodology given by Kono (1978) with slight modifications. 0.05ml sample, 1.5ml extraction buffer (50mM sodium carbonate buffer pH10.0), 0.5ml of 96µM NBT (Nitroblue tetrazolium), 0.1ml TritonX-100, 0.1ml of 20mM hydroxylamine hydrochloride were taken in cuvette and increase in absorbance was recorded at 540nm. The enzyme activity was expressed as µM/ml (haemolymph) and µM/mg (midgut) weight.

Glutathione- S-transferase (GST) activity:

GST activity was estimated using the method of Habig *et al.* (1974) with minor modifications. 50µl of 10mM CDNB (1- chloro-2, 4-dinitrobenzene), 100µl GSH (Reduced glutathione), 50µl of sample and 0.2ml of 0.1M sodium phosphate buffer containing PTU (phenylthiourea) were incubated at 25□C and change in absorbance was recorded at 340nm for 5min at 1min interval. The enzyme activity was expressed as µM/ml (haemolymph) and µM/mg (midgut) weight.

Effect on morphology of haemocytes:

To study the morphological alterations in haemocytes the methodology of Wang *et al*. (2012) with slight modifications was followed. Haemolymph of insects exposed for 96 hours was bled on termanox discs after cutting proleg of larvae. It was allowed to dry and fixed with 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2) for two hours. After this sequential dehydration was done by using graded series of ethanol i.e 25% followed by 50%, 70%, 90% and at the end with absolute (100%) alcohol. Then discs were placed in dry chamber for proper drying. At the end silver coating was done by mounting samples on aluminium stubs and haemocytes were observed under SEM at magnification of 10.00KX operated at 10KV. The percentage of cells showing various deformities were also calculated in treatment and control group after 96hr exposure to *S.commune* ethyl acetate extract.

Statistical analysis:

To study the effect of duration one way analysis of variance (ANOVA) with Tukey's test was performed and to study the effect of treatment student's t-test was applied.

Abbreviations

Catalase (CAT), Ascorbate peroxidase (APOX) , Superoxide dismutase (SOD), Glutathione- S-transferase (GST).

Declarations

The authors declare that they have no competing interest.

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Authors' contributions:

Pooja Chadha , Amarjeet Kaur and Snehdeep Kaur designed the study and analyzed the content. Mandeep Kaur performed the experiments and analyzed the content related to it. Rajvir Kaur helps in fungus identification as well as in isolation. All authors read and approved the final manuscripts.

Availability of data and materials:

All data generated or analyzed during this study are included in this article and its additional files.

Ethical approval and consent to participate:

Not applicable. This article does not contain any studies involving human participants or animals performed by any of the authors.

Consent for publication:

Not applicable.

Competing interests

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Tables

Table.1: Catalase activity in haemolymph and midgut of *S. litura* after treatment with ethyl acetate extract of *S. commune*.

Parameter	Groups	Exposure Time (hrs)				
		24	48	72	96	F value
Catalase Activity in haemolymph (µmole/ml)	Control	9.69±0.54^{a}	10.62±0.72^{ab}	13.75±1.08^{b}	14.06±0.90^{b}	6.934*
	EG(LC_{50})	34.06±1.24^{a}	43.43±0.93^{b}	52.18±2.81^{c}	51.87±0.36^{c}	63.04**
	t – value	17.72**	36.40**	19.69**	38.91**	
Catalase Activity in midgut (µmole/mg)	Control	12.18±0.90^{a}	14.37±1.08^{ab}	15.93±1.26^{ab}	17.18±0.56^{b}	4.798*
	EG(LC_{50})	25.94±1.26^{a}	33.75±1.44^{b}	52.18±1.98^{c}	66.56±1.98^{d}	116.06**
	t – value	8.85**	10.73**	15.41**	24.00**	

* ($p \leq 0.05$), ** ($p \leq 0.01$), EG=Exposed group. The values represented as mean ± standard error. Different letters a, b, c between the columns are significantly different (Tukey's test, $p \leq 0.05$) and signify the effect of duration.

Table.2: Ascorbate peroxidase activity in haemolymph and midgut of *S. litura* after treatment with ethyl acetate extract of *S. commune*

Parameter	Groups	Exposure Time (hrs)				
		24	48	72	96	F value
Ascorbate Peroxidase Activity in haemolymph (µmole/ml)	Control	24.15 ± 2.72^{a}	25.92 ± 2.72^{a}	27.69 ± 2.38^{a}	29.46 ± 2.72^{a}	NS
	EG(LC_{50})	81.31 ± 1.36^{a}	167.06 ± 3.57^{b}	184.15 ± 1.53^{c}	236.00 ± 2.89^{d}	652.72*
	t – value	18.79**	31.41**	55.26**	52.01**	
Ascorbate Peroxidase Activity in midgut (µmole/mg)	Control	45.07 ± 1.87^{a}	47.44 ± 1.87^{a}	50.38 ± 3.06^{a}	53.03 ± 3.06^{a}	NS
	EG(LC_{50})	109.60 ± 2.72^{a}	139.36 ± 3.57^{b}	209.26 ± 5.10^{c}	282.26 ± 5.10^{d}	411.58**
	t – value	19.52**	22.79**	35.68**	38.51**	

* ($p \leq 0.05$), ** ($p \leq 0.01$), NS= non-significant, EG=Exposed group. The values represented as mean ± standard error. Different letters a, b, c between the columns are significantly different (Tukey's test, $p \leq 0.05$) and signify the effect of duration.

Table.3: Superoxide dismutase (SOD) activity in haemolymph and midgut of *S. litura* after treatment with ethyl acetate extract of *S. commune*

Parameter	Groups	Exposure Time (hrs)				
		24	48	72	96	F value
SOD Activity in haemolymph (µmole/ml)	Control	28.31±0.18^{a}	28.46±0.10^{a}	28.55±0.21^{a}	28.82±0.31^{a}	NS
	EG(LC_{50})	31.20±0.14^{a}	32.41±0.31^{a}	37.36±0.42^{a}	44.35±0.62^{b}	208.79**
	t – value	12.29**	12.15**	18.55**	22.40**	
SOD Activity in midgut (µmole/mg)	Control	28.57±0.11^{a}	28.44±0.39^{a}	28.60±0.22^{a}	28.84±0.12^{a}	NS
	EG(LC_{50})	32.08±0.24^{a}	40.36±0.30^{ab}	45.83±1.20^{b}	119.63±3.49^{c}	474.91**
	t – value	13.24**	23.99**	14.07**	25.95**	

** ($p \leq 0.01$), NS= non-significant, EG=Exposed group. The values represented as mean ± standard error. Different letters a, b, c between the columns are significantly different (Tukey's test, $p \leq 0.05$) and signify the effect of duration.

Table.4: GST activity in haemolymph and midgut of *S. litura* after treatment with ethyl acetate extract of *S. commune*

Parameter	Groups	Exposure Time (hrs)				
		24	48	72	96	F value
GST Activity in haemolymph (µmole/ml)	Control	7.91±0.24^{a}	8.12±0.26^{a}	8.96±0.12^{a}	8.75±0.48^{a}	NS
	EG(LC_{50})	18.33±0.72^{a}	18.75±0.48^{a}	21.66±1.20^{a}	19.79±0.84^{a}	NS
	t – value	13.69**	17.71**	10.50**	11.38**	
GST Activity in midgut (µmole/mg)	Control	7.08±0.24^{a}	7.29±0.60^{ab}	8.96±0.12^{b}	8.95±0.36^{b}	NS
	EG(LC_{50})	9.16±0.48^{a}	18.12±0.60^{b}	30.00±0.72^{d}	26.46±0.84^{c}	189.03**
	t – value	3.86*	12.72**	28.75**	19.09**	

* ($p \leq 0.05$), ** ($p \leq 0.01$), NS= non-significant, EG=Exposed group. The values represented as mean ± standard error. Different letters a, b, c between the columns are significantly different (Tukey's test, $p \leq 0.05$) and signify the effect of duration.

Figures

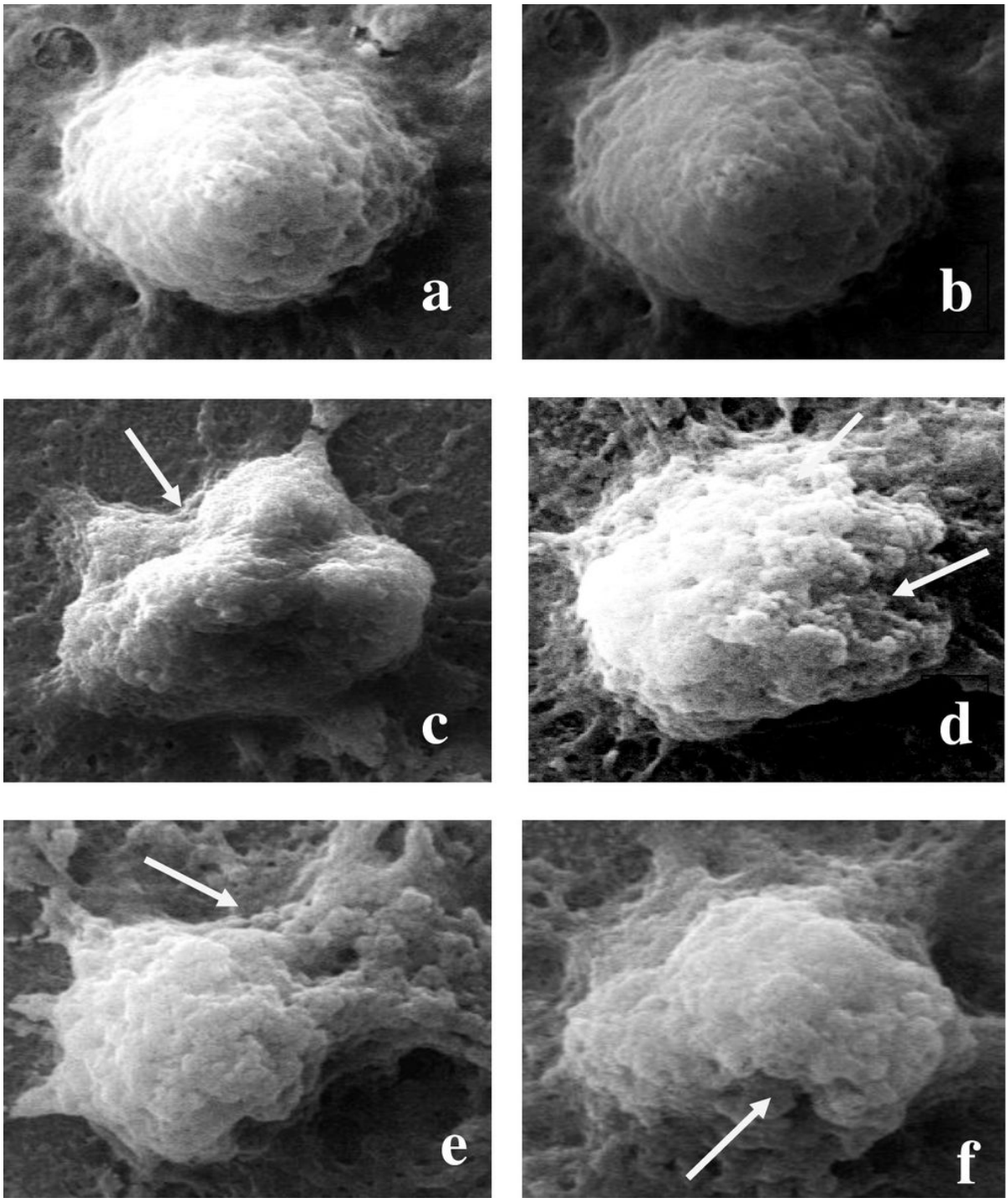

Figure 1

Microphotographs showing haemocytes (Plasmatocytes) (a-b). Normal haemocyte; (c-f). Various deformities observed in haemocytes after treatment with ethyl acetate extract of S. commune; (c). Cell membrane shrinkage of haemocyte; (d-f). Breakage in membrane and cytoplasmic leakage.

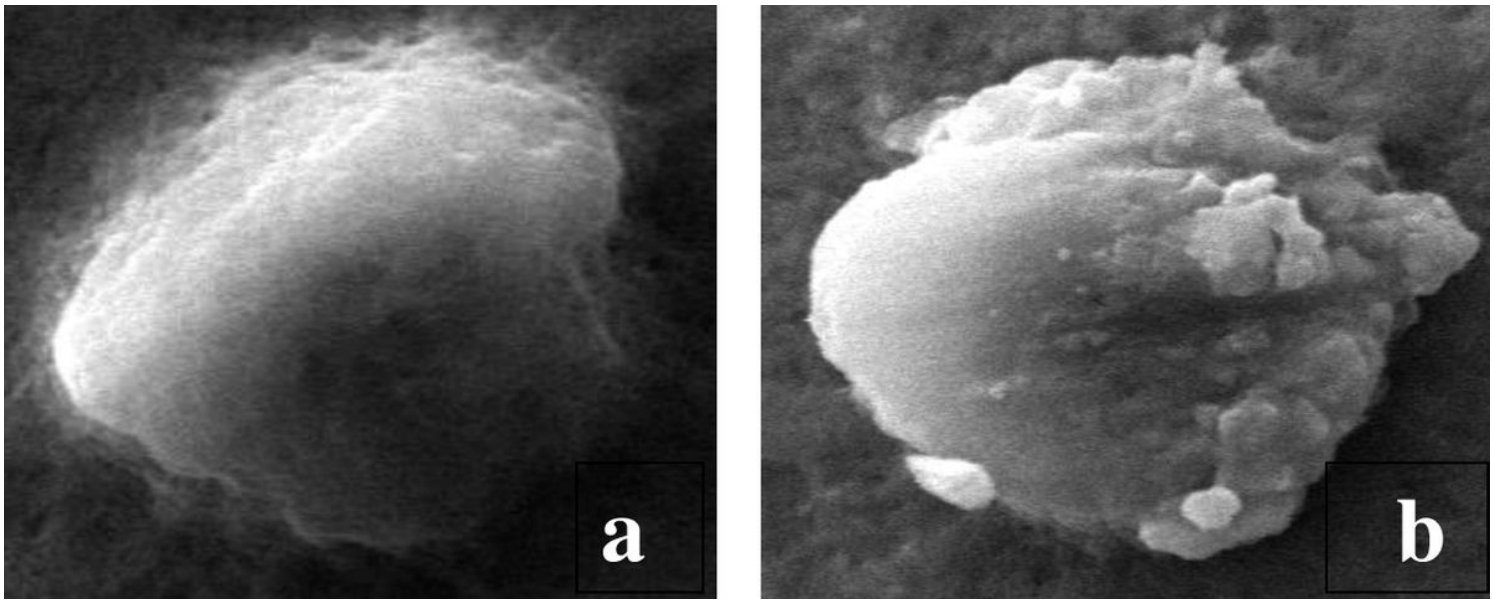

Figure 2

Microphotographs showing haemocytes (Granulocytes) (a). Normal haemocyte; (b). Strumae and surface abnormalities in haemocytes after treatment with ethyl acetate extract of S. commune

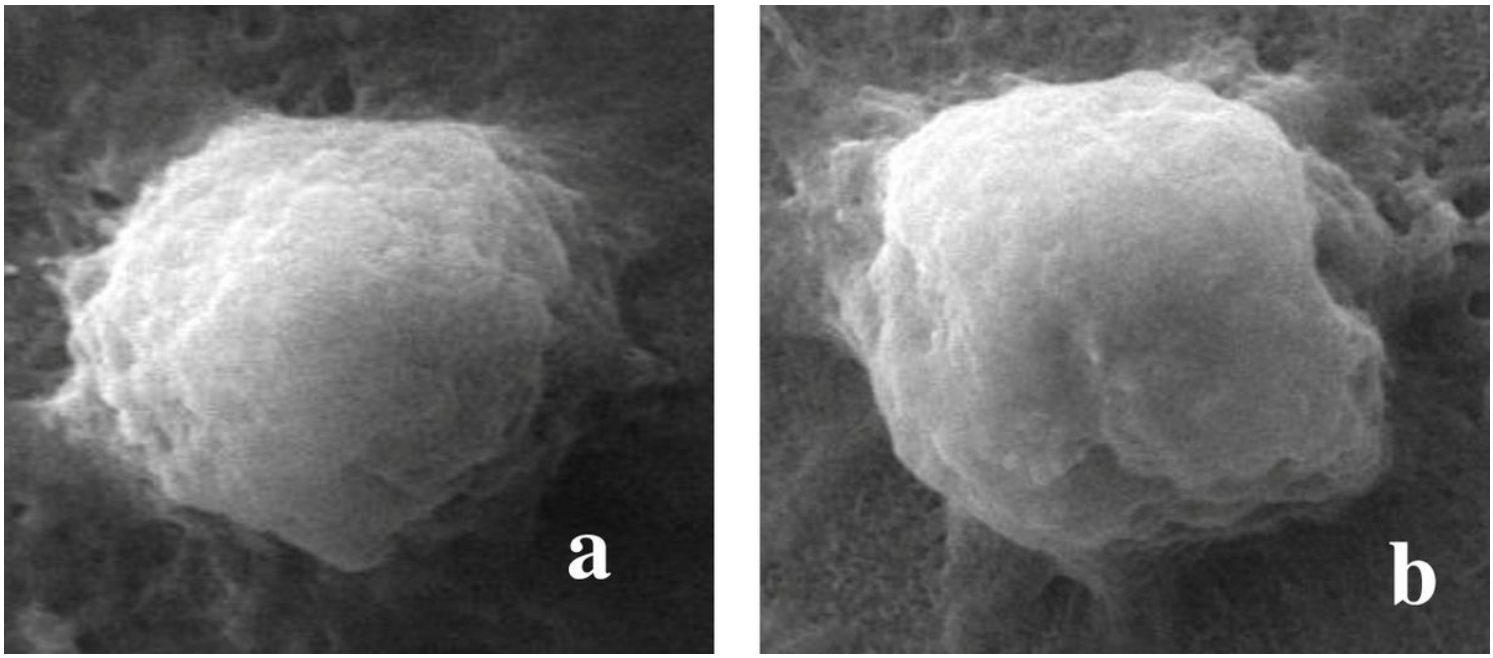

Figure 3

Microphotographs showing haemocytes (prohaemocyte) (a). Normal haemocytes (b). Cell membrane shrinkage in haemocyte after treatment with ethyl acetate extract of S. commune

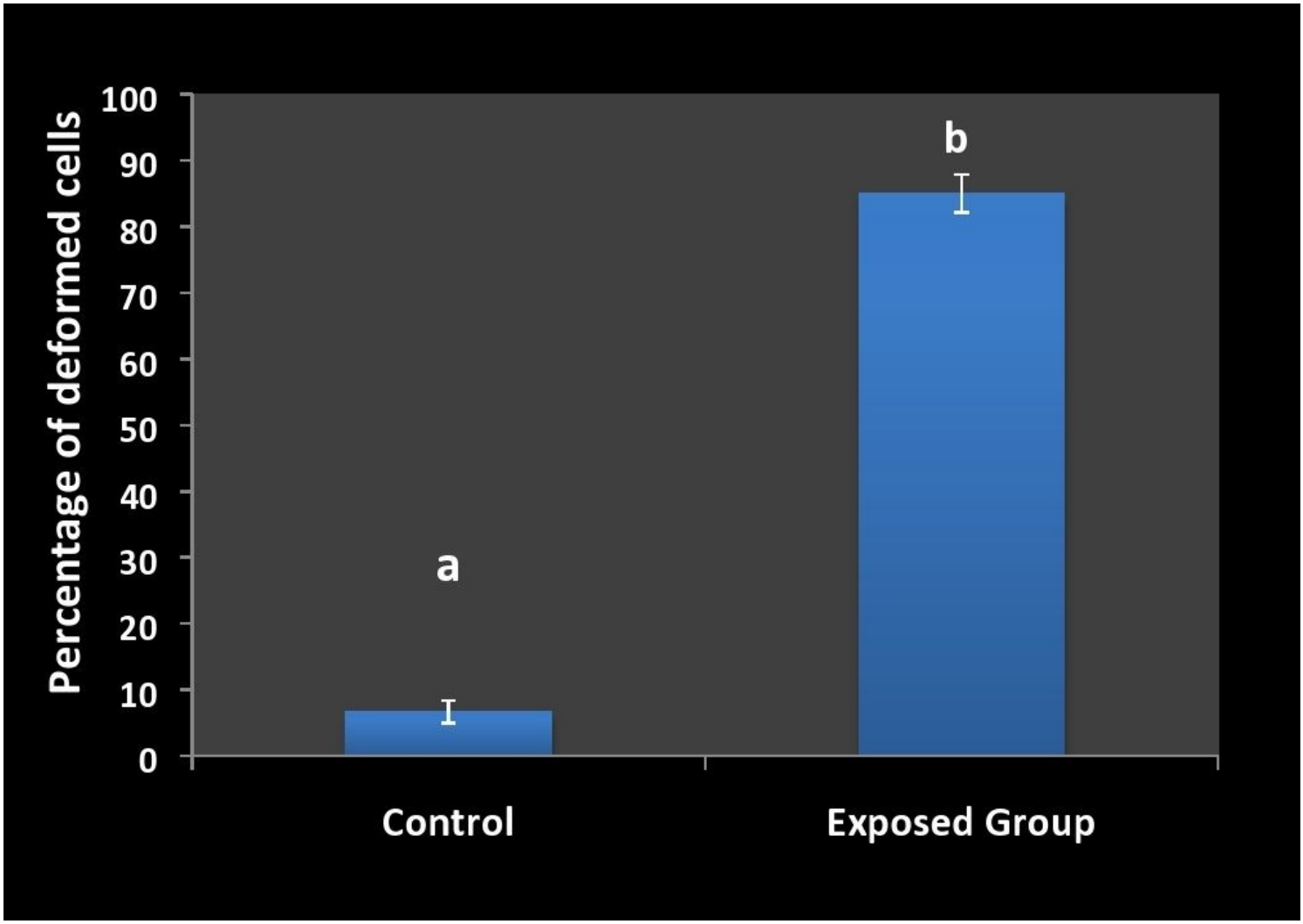

Figure 4

The percentage of cells showing various deformities

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