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Original Research Article

Expression of transaminase enzymes and effect of potassium iodide on its production in mycelial form of *Sporothrix schenckii*Rajesh Bareja^{1,*}, Pottathil Shinu²¹Dept. of Microbiology, Govt. Medical College, Badaun, Uttar Pradesh, India²Dept. of Biomedical Science, College of Clinical Pharmacy, King Faisal University, Al-Ahsa, Saudi Arabia

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ABSTRACT

Background : Sporotrichosis is chronic, pyogranulomatous fungal infection of cutaneous or subcutaneous. It is caused by the dimorphic fungus *Sporothrix schenckii* (*S. schenckii*).

Aims: This study describes the in-vitro effect of potassium iodide (KI) on the transaminases enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) produced by the filamentous form of *S. schenckii*.

Methods and Material: A master culture of *S. schenckii* was prepared in YNB (Yeast nitrogen base) medium and was incubated at 25°C (mould). KI was added into the YNB medium in increasing concentrations. One mL suspension of master culture was inoculated into each bottle and incubated at 25°C for different time period, 4th day (early-log period), 9th day (mid-log period) and 14th day (peak of growth) respectively. After centrifuging, a 5% homogenate was prepared that was used for transaminases enzyme assay.

Results: The mean aspartate aminotransferase level of control specimen was 12.4 ± 3.30, 22.4 ± 3.69 and 53.6 ± 8.46 IU on day 4, 9 and 14 respectively. The mean aspartate aminotransferase level of test specimen was ranged from 3.5 ± 0.80 (KI 6.4 gram %) to 12.4 ± 4.66 IU (KI 0.1 gram %), 2.6 ± 0.21 (KI 6.4 gram %) to 29.5 ± 7.31 IU (KI 0.05 gram %) and 2.5 ± 0.23 (KI 6.4 gram %) to 42.3 ± 3.70 IU (KI 0.2 gram %) on day 4, 9 and 14 respectively. The mean alanine aminotransferase level of control specimen was 17.5 ± 5.93, 24.6 ± 3.59 and 32.6 ± 7.54 IU on day 4, 9 and 14 respectively. The mean alanine aminotransferase level of test specimen was ranged from 2.2 ± 0.00 (KI 6.4 gram %) to 15.4 ± 2.36 IU (KI 0.1 gram %), 2.7 ± 0.81 (KI 6.4 gram %) to 29.5 ± 2.75 IU (KI 1.6 gram %) and 3.5 ± 1.37 (KI 6.4 gram %) to 29.6 ± 2.82 IU (KI 0.2 gram %) on day 4, 9 and 14 respectively.

Conclusions: At the entire test concentrations mean value was lower as compared to control. The low activity of the transaminases enzymes indicates that KI has inhibitory effect on the growth of *S. schenckii* (mould) and has led to decrease in the activity of these enzymes.

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1. Introduction

Sporothrix schenckii (*S. schenckii*), a dimorphic fungus, is distributed worldwide and known as the etiological agent of sporotrichosis. Various clinical manifestations of sporotrichosis are recognized viz. lymphocutaneous, localized cutaneous, disseminated, mucosal, skeletal and visceral.¹ Pathogenic potential of *S. schenckii* is not as much

known on mammalian host due to scarcity of enzymatic information. Transaminases or aminotransferases being a part of protein is found in various fungi. Fungal aspartate aminotransferase is very active in free-living mycelium.² Chalot et al.³ explained that synthesis of amino acid precursors during TCA cycle operation is an essential step for aspartate and alanine synthesis through aminotransferase activities in ectomycorrhizal fungus *Paxillus involutus*. No study has been found that shows the activity of transaminases enzymes, aspartate aminotransferase (AST)

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and alanine aminotransferase (ALT) in *S. schenckii*, the causative agent of sporotrichosis. Potassium iodide (KI) has been used in the treatment of sporotrichosis, however, the exact mechanism of action remains unknown.⁴ Therefore, it was planned to undertake the present study to estimate the transaminases enzymes in the mycelial form of *S. schenckii* and to study the effect of KI with increasing concentration on the production of these enzymes in-vitro.

2. Material and Methods

This experimental study was conducted in the department of Microbiology in a tertiary care hospital. A standard strain of *S. schenckii* (ATCC 14284 / MTCC 1359) was procured from Institute of Microbial Technology, Chandigarh, India. A master culture was prepared by doing the subculture of *S. schenckii* from slope of Sabouraud's dextrose agar (SDA) in 50 mL of YNB (Yeast nitrogen base, HiMedia, Mumbai) medium in a screw-capped bottle and was incubated at 25°C. On the seventh day the suspension of YNB medium with *S. schenckii* was adjusted to 90% transmission at 540 nm on the photo-colorimeter.⁴ Master culture thus prepared was used for subsequent analysis.

YNB medium was prepared and dispensed in 50 mL aliquots into 150 screw capped (160 mL capacity) bottles. Potassium iodide was added into the YNB medium in increasing concentrations in such a way so as to have final concentrations of 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 gram percent of the medium. One bottle of YNB without KI was served as a control. Out of 150 bottles, 50 bottles each were used for three different days, i.e. 4th day (early-log period), 9th day (mid-log period), 14th day (peak of growth). Out of 50 bottles, 5 bottles were used as control (without KI) and rest were used for various concentration of KI. To reduce the error 5 bottles were used for each concentration of KI. One mL suspension of master culture was inoculated into each bottle and incubated at 25°C for different time period respectively. The content of respective bottle was centrifuged at 6000 rpm for half an hour on the 4th, 9th and 14th day. The deposit thus obtained was suspended in 5.0 mL citrate buffer saline (0.15 mol/L Sodium chloride, 0.015 mol/L Sodium citrate, pH 7) and was centrifuged again at 6000 rpm for half an hour. The process was repeated twice to ensure proper washing. The deposit thus obtained was taken, dried in the folds of filter paper, weighed and was crushed finely in a tissue homogenizer. A 5% homogenate was prepared from each weighed tissue in ice-cold distilled water and was used for subsequent enzyme assay.^{1,4} The transaminases enzymes, aspartate aminotransferase and alanine aminotransferase were determined by the method of Reitman and Frankel.⁵ The enzymes AST and ALT were estimated in per mg wt. of homogenate by following formula:

2.1. Calculation

$$AST/ALT(I.U/L) = \frac{T-C}{S} \times 16.1 \times \frac{\text{Vol. of homogenate}}{0.2 \text{ ml}} \times \frac{1}{\text{Wt. of tissue}}$$

2.2. Statistical analysis

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. ANOVA (Analysis of Variance) test was used to compare the within group and between group variances amongst the study groups. Dunnett's "t"-Test was used for comparing each experimental mean with the control mean.

3. Results

The enzymes aspartate aminotransferase and alanine aminotransferase were determined for three different days, 4th day (early-log period), 9th day (mid-log period) and 14th day (peak of growth) respectively.

3.1. (a) Aspartate aminotransferase (AST)

On day 4, an irregular yet generally regressive trend of mean aspartate aminotransferase values was observed with increasing concentrations. The trend was polynomial in nature with higher values at blank, KI 0.1 and 1.6 gram % and values were of middle order at KI 0.05, 0.2, 0.4 and 0.8 gram % concentrations. The values were of lower order at KI 3.2 and 6.4 gram % concentrations (Table 1). On day 9, a generalized declining trend of aspartate aminotransferase values was observed with increasing concentration. Mean values were 22.42 and 29.51 IU at blank and KI 0.05 gram % which declined to reach at 3.44 and 2.63 IU at KI 3.2 and 6.4 gram % concentrations respectively (Table 2). On day 14, a generalized declining trend of aspartate aminotransferase values was observed with increasing concentration. Mean values were 53.57 and 38.54 IU at blank and KI 0.05 which declined to reach at 5.41 and 2.55 IU at KI 3.2 and 6.4 gram % concentrations respectively (Table 3).

On day 4, mean aspartate aminotransferase level of control specimen was 12.4 ± 3.30 IU. The aspartate aminotransferase level of test specimens was ranged from 3.5 ± 0.80 (KI 6.4 gram %) to 12.4 ± 4.66 IU (KI 0.1 gram %) (Table 4). On day 9, mean aspartate aminotransferase level of control specimen was 22.4 ± 3.69 IU. Among different test concentrations mean aspartate aminotransferase levels were ranged from 2.6 ± 0.21 (KI 6.4 gram %) to 29.5 ± 7.31 IU (KI 0.05 gram %) (Table 4). On day 14, mean aspartate aminotransferase level of control specimen was 53.6 ± 8.46 IU. For test specimens, mean values were ranged from 2.5 ± 0.23 (KI 6.4 gram %) to 42.3 ± 3.70 IU (KI 0.2 gram %) (Table 4). By comparing all the three days, in general there was an increase in the activity of

enzyme with increase in duration of incubation 4th to 14th day (Table 5). The mean value was lower significantly for all the test concentrations as compared to control. No deposit obtained at the concentration KI 12.8 gram%.

3.2. (b) Alanine aminotransferase (ALT)

On day 4, an irregular but regressive trend of mean alanine aminotransferase levels with increasing concentration was observed with higher mean values at blank and KI 0.1 concentration and lower at KI 3.2 and 6.4 gram % concentrations (Table 6). On day 9, an irregular but regressive trend of alanine aminotransferase levels with increasing concentration was observed with higher mean values at KI 1.6 and 0.1 gram % and lower at KI 3.2 and 6.4 gram % concentrations (Table 7). On day 14, an irregular but declining trend of alanine aminotransferase levels was observed with increasing concentrations with peak values at blank and KI 0.2 gram % and minimum values at KI 3.2 and 6.4 gram % concentrations (Table 8).

On day 4, mean alanine aminotransferase level of control specimen was 17.5 ± 5.93 IU. The alanine aminotransferase levels of test specimen were ranged from 2.2 ± 0.00 (KI 6.4 gram %) to 15.4 ± 2.36 IU (KI 0.1 gram %) (Table 9). On day 9, mean alanine aminotransferase level of control specimen was 24.6 ± 3.59 IU. Among different test concentrations mean alanine aminotransferase levels were ranged from 2.7 ± 0.81 (KI 6.4 gram %) to 29.5 ± 2.75 IU (KI 1.6 gram %) (Table 9). On day 14, mean alanine aminotransferase level of control specimen was 32.6 ± 7.54 IU. For test specimens, mean values were ranged from 3.5 ± 1.37 (KI 6.4 gram %) to 29.6 ± 2.82 IU (KI 0.2 gram %) (Table 9). By comparing all the three days, an increase in mean alanine aminotransferase levels of control together with all test concentrations was observed (Table 10). The mean value of test specimens was lower significantly for all the test concentrations as compared to control. No deposit obtained at the concentration KI 12.8 gram%.

4. Discussion

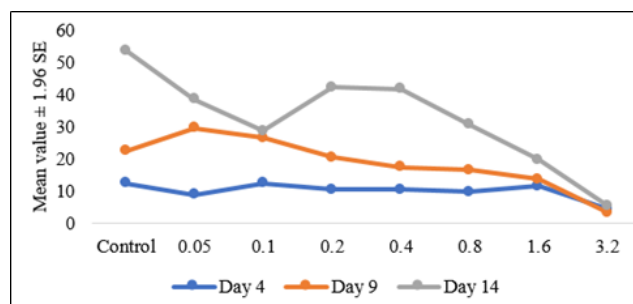
Transamination is a process in which an amino group is transferred from an amino acid to an alpha-ketoacid. It is an important step in the metabolism of amino acids. Amino acids are synthesized in tissues by amination of ketoacids derived from carbohydrate, protein and fat metabolism. The enzymes responsible for transamination are called transaminases or aminotransferases. Botton and Dell⁶ had determined the glutamate dehydrogenase and aspartate aminotransferase enzyme activities in various fungal isolates (*Laccaria laccata*, *Hebeloma westraliense*, *Scleroderma verrucosum* and *Pisolithus tinctorius*) by electrophoresis when the fungal cultures were in the exponential growth phase, after 10-15 days incubation. In addition, two electrophoretic isozyme patterns, esterase and

glutamate oxalate transaminase (GOT), were determined to measure variability among 10 isolates of *Fusarium* species. The activity of GOT was observed in the samples of *Fusarium solani* and *Fusarium oxysporum* and not detected in other isolates of *Fusarium*.⁷ They studied the activity of transaminases enzyme in several fungus. Neither they studied the activity of transaminases in *S. schenckii* nor studied the effect of KI with varied concentration on it.

In the present study, the activity of transaminases enzymes AST and ALT was estimated on 4th, 9th and 14th day of incubation for mycelial form respectively that included various phases of growth i.e. early log, mid log and exponential phase.

4.1. (a) Aspartate aminotransferase (AST)

In themycelial phase, on day 4, mean aspartate aminotransferase level of control specimen was 12.4 ± 3.30 IU. At all the test concentrations mean value was lower than control. Statistically, significant difference in mean AST levels as compared to control was observed only at KI 3.2 and 6.4 gram % ($p < 0.05$) (Table 4). On day 9, mean aspartate aminotransferase level of control specimen was 22.4 ± 3.69 IU. A continuous decline was observed at all the test concentrations and this decline was statistically insignificant ($p > 0.05$) for concentrations of KI 0.05, 0.1, 0.2, 0.4, and 0.8 gram %. A significant difference between test groups and control group was observed at concentrations of KI 1.6, 3.2 and 6.4 gram % respectively (Table 4). On day 14, mean aspartate aminotransferase level of control specimen was 53.6 ± 8.46 IU. A decline in mean AST for concentrations of KI 0.05 and 0.1 gram % followed by instant incline at KI 0.2 gram % and again followed by a continuous decrease in mean AST level was observed. Mean value of test specimens was significantly lower as compared to control for all the test concentrations (Table 4 & Graph 1).



Graph 1: Expression of mean aspartate aminotransferase (AST) in *S. schenckii* (mould) at different time intervals

By comparing all the three days, in general there was an increase in the activity of enzyme aspartate aminotransferase with increase in duration of incubation 4th to 14th day except concentration of KI 6.4 gram %.

Table 1: Expression of AST in *S. schenckii* (mould) on 4th day

Conc. of KI (gram %)	I	II	III	IV	V	Mean (IU)
Control	12.24	10.44	14.66	16.42	8.12	12.37
0.05	8.66	10.24	11.56	4.62	9.22	8.86
0.1	12.44	15.34	8.64	7.22	18.50	12.42
0.2	10.62	7.42	9.24	9.44	15.82	10.50
0.4	10.20	9.36	6.50	12.82	13.22	10.42
0.8	9.24	14.36	10.66	5.84	8.22	9.66
1.6	11.56	8.44	13.64	10.22	13.42	11.45
3.2	4.62	2.22	5.34	5.62	4.22	4.40
6.4	3.44	2.34	4.54	3.82	3.42	3.51

Table 2: Expression of AST in *S. schenckii* (mould) on 9th day

Conc. of KI (gram %)	I	II	III	IV	V	Mean (IU)
Control	22.24	26.42	25.62	20.3	17.56	22.42
0.05	29.42	20.34	34.56	24.72	38.52	29.51
0.1	26.42	25.64	28.32	30.62	21.54	26.50
0.2	20.46	20.82	17.34	19.86	24.24	20.54
0.4	17.24	16.48	21.66	19.48	12.38	17.44
0.8	16.46	18.82	20.64	11.42	15.62	16.59
1.6	15.32	17.46	21.46	1.82	12.36	13.68
3.2	3.26	2.22	4.64	3.84	3.26	3.44
6.4	2.48	2.86	2.48	2.86	2.48	2.63

Table 3: Expression of AST in *S. schenckii* (mould) on 14th day

Conc. of KI (gram %)	I	II	III	IV	V	Mean (IU)
Control	53.46	60.66	43.84	63.24	46.66	53.57
0.05	38.66	43.82	35.44	40.28	34.5	38.54
0.1	28.4	25.68	30.82	24.76	33.84	28.70
0.2	42.34	39.54	46.44	37.84	45.5	42.33
0.4	41.44	44.82	39.64	37.68	44.82	41.68
0.8	30.46	35.68	29.84	32.64	24.44	30.61
1.6	19.82	24.64	21.36	15.44	17.24	19.70
3.2	5.48	3.68	2.16	7.24	8.5	5.41
6.4	2.22	2.46	2.64	2.82	2.64	2.55

Table 4: Expression of mean AST in *S. schenckii* (mould) at different time intervals (n=5 for each concentration)

Conc. of KI (gram %)	Day 4			Day 9			Day 14		
	Mean (IU)	SD	"p"	Mean (IU)	SD	"p"	Mean (IU)	SD	"p"
Control	12.4	3.30		22.4	3.69		53.6	8.46	
0.05	8.9	2.61	0.285	29.5	7.31	0.074	38.5	3.77	<0.001
0.1	12.4	4.66	1.000	26.5	3.38	0.545	28.7	3.73	<0.001
0.2	10.5	3.18	0.867	20.5	2.47	0.981	42.3	3.70	0.001
0.4	10.4	2.74	0.840	17.4	3.48	0.334	41.7	3.16	0.001
0.8	9.7	3.16	0.556	16.6	3.50	0.191	30.6	4.14	<0.001
1.6	11.5	2.19	0.997	13.7	7.41	0.017	19.7	3.58	<0.001
3.2	4.4	1.34	0.001	3.4	0.89	<0.001	5.4	2.57	<0.001
6.4	3.5	0.80	<0.001	2.6	0.21	<0.001	2.5	0.23	<0.001

Significance of difference as compared to control (Dunnett's t-test has been used)

Table 5: Comparison of change in mean AST levels in *S. schenckii* (mould) at different concentrations

Conc. of KI (gram %)	Day 4 to Day 9			Day 4 to Day 14			Day 9 to Day 14		
	Mean Change (IU)	SD	"p"	Mean Change (IU)	SD	"p"	Mean Change (IU)	SD	"p"
Control	10.05	4.31	0.006	41.20	8.13	<0.001	31.14	8.94	0.001
0.05	20.65	6.93	0.003	29.68	5.10	<0.001	9.03	11.05	0.142
0.1	14.08	7.97	0.017	16.27	4.26	0.001	2.19	6.55	0.496
0.2	10.04	2.11	<0.001	31.82	3.37	<0.001	21.79	4.41	<0.001
0.4	7.03	5.66	0.050	31.26	3.94	<0.001	24.23	6.32	0.001
0.8	6.93	2.09	0.002	20.95	3.87	<0.001	14.02	5.25	0.004
1.6	2.23	7.13	0.523	8.24	4.80	0.018	6.02	5.00	0.055
3.2	-0.96	0.67	0.034	1.01	2.69	0.449	1.97	2.87	0.200
6.4	-0.88	0.92	0.099	-0.96	0.73	0.043	-0.08	0.25	0.535

Paired 't'-test used

Table 6: Expression of ALT in *S. schenckii* (mould) on 4th day

Conc. of KI (gram %)	I	II	III	IV	V	Mean (IU)
Control	17.24	25.46	13.82	20.78	10.22	17.50
0.05	9.62	6.72	7.52	11.22	8.62	8.74
0.1	14.22	16.28	18.82	15.3	12.5	15.42
0.2	13.42	12.64	17.34	14.22	9.66	13.45
0.4	12.34	14.56	9.66	11.82	8.22	11.32
0.8	11.22	9.86	5.32	7.42	8.44	8.45
1.6	12.34	15.88	8.44	15.88	10.22	12.55
3.2	3.28	4.24	3.28	2.82	5.64	3.85
6.4	2.24	2.24	2.24	2.24	2.24	2.24

Table 7: Expression of ALT in *S. schenckii* (mould) on 9th day

Conc. of KI (gram %)	I	II	III	IV	V	Mean (IU)
Control	24.48	29.46	21.82	20.66	26.74	24.63
0.05	22.64	27.48	31.34	28.48	32.66	28.52
0.1	28.54	33.48	35.56	23.84	21.82	28.64
0.2	15.54	17.48	20.66	24.48	19.24	19.48
0.4	20.34	11.52	16.88	19.84	14.64	16.64
0.8	22.76	20.28	17.54	27.48	24.62	22.53
1.6	26.48	27.62	29.24	33.46	30.88	29.53
3.2	4.44	2.64	2.64	6.46	6.46	4.52
6.4	2.84	2.84	3.46	2.84	1.28	2.65

Table 8: Expression of ALT in *S. schenckii* (mould) on 14th day

Conc. of KI (gram %)	I	II	III	IV	V	Mean (IU)
Control	32.46	41.76	28.22	22.76	37.82	32.60
0.05	14.34	20.82	16.24	23.56	11.82	17.35
0.1	25.76	21.24	18.36	26.78	15.76	21.58
0.2	28.76	30.42	29.82	33.34	25.54	29.57
0.4	25.54	24.28	20.82	27.62	29.76	25.60
0.8	9.64	12.42	14.82	7.32	18.72	12.58
1.6	13.24	19.76	12.54	16.32	20.68	16.50
3.2	5.82	3.46	3.46	8.24	6.42	5.48
6.4	3.46	2.48	2.48	5.82	3.46	3.54

(n=5 for each concentration)

Table 9: Expression of mean ALT in *S. schenckii* (mould) at different time intervals

Conc. of KI (gram %)	Day 4			Day 9			Day 14		
	Mean (IU)	SD	"p"	Mean (IU)	SD	"p"	Mean (IU)	SD	"p"
Control	17.5	5.93		24.6	3.59		32.6	7.54	
0.05	8.7	1.77	<0.001	28.5	3.90	0.404	17.4	4.78	<0.001
0.1	15.4	2.36	0.803	28.6	5.93	0.370	21.6	4.71	0.001
0.2	13.5	2.77	0.171	19.5	3.39	0.150	29.6	2.82	0.806
0.4	11.3	2.46	0.011	16.6	3.68	0.008	25.6	3.39	0.074
0.8	8.5	2.26	<0.001	22.5	3.84	0.915	12.6	4.45	<0.001
1.6	12.6	3.34	0.060	29.5	2.75	0.186	16.5	3.69	<0.001
3.2	3.9	1.13	<0.001	4.5	1.91	<0.001	5.5	2.05	<0.001
6.4	2.2	0.00	<0.001	2.7	0.81	<0.001	3.5	1.37	<0.001

Significance of difference as compared to control (Dunnett's t-test has been used)

Table 10: Comparison of change in mean ALT levels in *S. schenckii* (mould) at different concentrations

Conc. of KI (gram %)	Day 4 to Day 9			Day 4 to Day 14			Day 9 to Day 14		
	Mean Change (IU)	SD	"p"	Mean Change (IU)	SD	"p"	Mean Change (IU)	SD	"p"
Control	7.13	6.15	0.061	15.10	9.09	0.021	7.97	4.04	0.012
0.05	19.78	4.68	0.001	8.62	4.70	0.015	-11.16	6.64	0.020
0.1	13.22	4.08	0.002	6.16	5.27	0.059	-7.07	7.89	0.116
0.2	6.02	3.69	0.022	16.12	2.53	<0.001	10.10	2.94	0.002
0.4	5.32	4.72	0.065	14.28	4.66	0.002	8.96	4.82	0.014
0.8	14.08	3.98	0.001	4.13	5.47	0.166	-9.95	6.84	0.031
1.6	16.98	4.00	0.001	3.96	4.00	0.092	-13.03	4.04	0.002
3.2	0.68	2.00	0.491	1.63	2.44	0.210	0.95	0.69	0.036
6.4	0.41	0.81	0.320	1.30	1.37	0.100	0.89	1.67	0.300

Paired 't'-test used

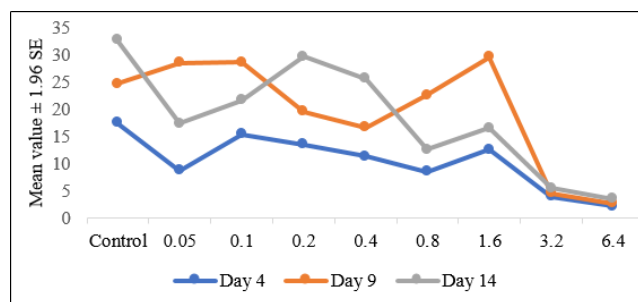
Between day 4 and day 9, an increase in mean aspartate aminotransferase levels of control as well as all test concentrations except KI 3.2 and 6.4 gram % was observed. This change was significant statistically too for all control as well as all the test concentrations except KI 1.6 and 6.4 gram % (Table 5). Between day 4 and 14, an increase in mean aspartate aminotransferase levels ($p < 0.05$) was observed for control as well as all the test concentrations except KI 6.4 gram %. The mean change was ranged from -0.96 ± 0.73 to $(KI\ 6.4\ gram\ \%)$ to 41.20 ± 8.13 IU (control). The change was significant statistically too for all the concentrations except KI 3.2 gram %. Between day 9 and day 14 too, an increase in mean aspartate aminotransferase levels was observed for control and all the test groups except KI 6.4 gram %. At concentration of KI 6.4 gram %, a decline in mean AST levels was observed during the period. The change was also significant statistically for control, KI 0.2, 0.4 and 0.8 gram % (Table 5).

4.2. (b) Alanine aminotransferase (ALT)

In the mycelial phase, on day 4, mean alanine aminotransferase level of control specimen was 17.5 ± 5.93 IU. For all test concentrations mean value was lower

as compared to control. Statistically, significant difference in mean alanine aminotransferase levels as compared to control was observed at concentration of KI 0.05, 0.4, 0.8, 3.2 and 6.4 gram % respectively ($p < 0.05$) (Table 9). On day 9, mean alanine aminotransferase level of control specimen was 24.6 ± 3.59 IU. A continuous decrease in the mean levels was observed in test concentrations from KI 0.05 gram % to onward except KI 0.8 and 1.6 gram %. A significant difference between test groups and control group was observed at concentrations of KI 0.4, 3.2 and 6.4 gram % respectively. On day 14, mean alanine aminotransferase level of control specimen was 32.6 ± 7.54 IU. A continuous increase in the mean test level was observed for KI 0.05, 0.1 and 0.2 gram % followed by a decrease in the mean test values. Mean value of test specimens was lower as compared to control for all the test concentrations (Table 9 & Graph 2).

By comparing all the three days, in general there was an increase in the activity of enzyme alanine aminotransferase with increase in duration of incubation 4th to 14th day in control and test concentrations except KI 0.05, 0.1, 0.8 and 1.6 gram % (Table 10). Between day 4 and day 9, an increase in mean alanine aminotransferase levels of control as well as all test concentrations was observed. This change



Graph 2: Expression of mean alanine aminotransferase (ALT) in *S.schenckii* (mould) at different time intervals

was significant statistically too for all the groups except control, KI 0.4, 3.2 and 6.4 gram %. Between day 4 and 14, an increase in mean alanine aminotransferase levels was observed for control as well as all the test concentrations. The change was significant statistically too for control, KI 0.05, 0.2 and 0.4 gram %. Between day 9 and day 14 too, an increase in mean alanine aminotransferase levels was observed. The change was also significant statistically for all the groups except KI 0.1 and 6.4 gram % (Table 10).

5. Conclusion

In this study, an increase in mean aspartate and alanine aminotransferase levels of control as well as all test concentrations was observed but at all these concentrations mean value of test concentrations was lower as compared to control. It indicates that KI has inhibitory effect on the growth of *S. schenckii* and this has led to decrease in activity of transaminases. This effect along with other defense mechanisms of the body may be the mode of action of KI in the treatment of sporotrichosis.

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8. Conflict of Interest

None.

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