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Management of heat stress in *Drosophila melanogaster* with *Abhrak bhasma* and ascorbic acid as antioxidant supplements

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ABSTRACT

Global warming and climate change are the most serious consequences of anthropogenic activities. Although global warming poses threat to all living organisms, poikilotherms are most vulnerable among them. In the current study, management of heat stress in insects that constitute the largest group of poikilotherms has been studied using *Drosophila melanogaster* as a model. Various catalytic and non-catalytic oxidative parameters, at physiological as well as molecular level, were analyzed after exposing the *Drosophila* flies to high temperature. The efficacy of *Abhrak bhasma* and ascorbic acid in ameliorating the heat stress was also investigated. It was observed that heat stress alters various parameters indicating oxidative stress. Heat stress influences the activity of superoxide dismutase and catalase enzymes and also has an effect on total reduced glutathione (GSH) content as well as GSH: oxidized form of GSH (GSSG) ratio. Dietary supplement of *Abhrak bhasma* and ascorbic acid was found to alter the changes in antioxidant parameters induced due to heat stress. These flies also showed an increase in expression of cap "n" collar C and heat shock protein 70 genes, which play a crucial role in the management of stressful conditions. Overall, supplementation of diet with *Abhrak bhasma* and ascorbic acid was found to boost the capacity of *Drosophila* to counter the effects of heat stress.

1. INTRODUCTION

Carbon emission has steadily gone up in every part of the globe over the past few decades. This has led to a steady rise in the environmental temperature [1]. With the dramatic changes in global temperature, organisms undergo changes at physiological and molecular levels. Various reports have indicated that exposure of an organism to heat results in the generation of an oxidative stress [2,3]. Heat stress brings about physiological changes in an organism such that the antioxidant mechanisms fail to match the rate of production of oxidants. The oxidative damage starts accumulating and increasingly severe symptoms appear in an organism till it succumbs to them. This is one of the reasons for shorter life expectancy of the ecotypes of a species in the habitats with higher temperature [4]. It is rewarding to comprehend these physiological as well as molecular changes due to rise in ambient temperature.

Life is sustained due to the inherent antioxidant mechanisms that strive to outdo the production of reactive oxygen species (ROS) and other free radicals in response to stresses. Organisms manage the oxidative stress mainly through non-catalytic and catalytic pathways [5,6]. The non-catalytic pathway targets ROS and other free radicals in a non-specific manner while the catalytic route specifically targets free radicals and molecules through various enzymes.

Insects have been playing a prominent role in the survival of human species, in positive as well as negative ways. Most insects are quite sensitive to temperature of the surrounding. Their distribution, life expectancy, activity patterns, reproduction, and several other biological aspects are influenced by the temperature of the place and time [7]. Thus, change in global temperature can jeopardize the survival of many insect species while promoting the propagation of others. *Drosophila melanogaster* flies have been widely used as a model system not only to understand the responses in insect group but also to validate many aspects that have direct implications in human beings.

Supplementing the diet of an organism with natural or synthetic antioxidants can strengthen the inherent antioxidant capacity, to counter the effects of stress [8,9]. These antioxidants may be in the form of vitamins and polyphenols such as flavonoids, which directly or indirectly take part in scavenging of free radicals [10]. Apart from the naturally occurring compounds, various synthetic products have been used as antioxidant diet supplements. Traditional medicines, such as Ayurvedic preparations, have shown promising scope in management of oxidative stress [3]. Bhasma are a type of time-tested Ayurvedic preparations made by repeated incineration of minerals

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mixed with decoction of medicinal plants. *Abhrak bhasma* is a type of *bhasma* prepared from mineral mica incinerated a certain number of times (*puta*) with decoction of about 70 different medicinal herbs. The quality and efficacy of *Abhrak bhasma* depends mainly on the cycles or *putas* being performed. Sahastra puti (involving 1000 incineration cycles) *Abhrak bhasma* is considered to be of the finest quality, having capacity to reduce the effects of oxidative stress and is employed in the treatment of a great variety of diseases [11].

The current study reports the effects of heat stress on various parameters, indicating oxidative stress, in *D. melanogaster*. This study also explores the efficacy of *Abhrak bhasma* in comparison with ascorbic acid, a known antioxidant, in amelioration of the effect of heat stress in *Drosophila*.

2. MATERIALS AND METHODS

2.1. *Abhrak Bhasma* Source

Sahastra puti *Abhrak bhasma* was procured from Dhootapapeshwar Ltd., among the leading manufacturers of ayurvedic medicines (Batch no: P150300110).

2.2. Drosophila Husbandry

D. melanogaster (Canton S strain) flies were maintained on corn-meal agar medium at 26°C under 12 h light-12 h dark cycles. The control flies were allowed to lay eggs on culture media containing 0% AB, 0.1% AB, 0.5% AB, and 20 mM ascorbic acid for 12 h. After 12 h, the adults were removed, and the eggs were allowed to develop till they attained 2 days of adult age. These flies were then subjected to heat stress.

2.3. Heat stress

In three replicates, 160 2-day-old flies each, from 0% AB, 0.1% AB, 0.5% AB, and 20-mM ascorbic acid feeding regimes, were removed and segregated into control and test groups. The flies of control group were maintained in a chamber set at a temperature of 26°C, whereas the flies of test group were maintained in a chamber with a temperature of 38°C for 1 h, under the condition of same humidity and illumination. After 1 h, the flies from each group were separated into vials with (a) Trizolin for RNA extraction (10 flies each), (b) protein extraction buffer for catalase and superoxide dismutase (SOD) enzyme assays (20 flies each), (c) reduced glutathione (GSH) assay extraction buffer (20 flies each), and (d) 0.154 M KCl extraction for 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and lipid peroxidation assay (26 flies each). Equal numbers of male and female flies were maintained in all the vials. All the estimations were carried out in reference to the amount of protein used per reaction. The protein content of each extract was measured using Bradford's assay [12]. The final results were represented as percent change in comparison to the control flies not subjected to heat stress and shown as baselines of activity or concentration in each graph (except for polymerase chain reaction [PCR] results).

2.4. Catalase Assay

Catalase assay was performed by monitoring molecular breakdown of H_2O_2 by catalase at 240 nm in pH 7 as per the method described by Aebi [13]. The adults were homogenized in protein extraction buffer containing 20 mM tris-acetate buffer (pH 7.8), 0.1% triton X-100, and 1mM PMSF, using micropestle homogenizer (Sigma catalog no: Z359955-1EA). The tissue extract was then added to 0.1 M phosphate buffer containing 17 mM H_2O_2 and its degradation was immediately followed at 240 nm on Biotek spectrophotometer (Model no: EPOCH–Gen5). One unit of catalase activity was defined as enzyme required for decomposing 1.0 µmole of H_2O_2 per minute at pH 7.0 at 25°C and expressed as enzyme units.

2.5. SOD Assay

SOD assay was performed by pyrogallol auto-oxidation inhibition method as described by Marklund and Marklund [14] with slight modifications. The method involves blocking of the auto-oxidation of pyrogallol by SOD. The inhibition of oxidation of pyrogallol was followed at 420nm on a Biotek spectrophotometer (Model no: EPOCH–Gen5). One unit of SOD activity was defined as amount of the enzyme that reduces pyrogallol auto-oxidation by 50% at pH 8.5 at 25°C and expressed as enzyme units.

2.6. Glutathione Measurement (Total GSH and GSH:GSSG Ratio)

GSH and its oxidized form GSSG were measured by enzymatic recycling method using the protocol described by Rahman *et al.* (2006) [15]. The adult flies were homogenized in glutathione extraction buffer containing 5-mM EDTA (pH 7.5), 6 mg/ml sulfosalicyclic acid and 0.1% triton X-100 in 0.1 M phosphate buffer. Oxidation of GSH was carried out with 5, 5'-dithio-bis-(2-nitrobenzoic acid) to form a yellow derivative, 5-thio-2-nitro-benzoic acid (TNB), measurable at 412 nm. The GSSG, thus formed, is recycled using glutathione reductase enzyme in the presence of nicotinamide adenine dinucleotide phosphate. The rate of formation of TNB in comparison to standard was correlated to find the concentration of the GSH and GSSG in samples. The free GSH was derivatized with 2-vinylpyridine for the accurate measurement of GSSG. The excess of 2-vinylpyridine was later neutralized with triethanolamine. The GSH and GSSG content were measured as nM/mg of protein.

2.7. DPPH Scavenging Assay

Free-radical scavenging capacity of tissue was measured using DPPH free-radical scavenging assay [16]. The adults (15 males and 15 females) were homogenized in 0.154M KCl using micropestle homogenizer, and the whole body extract was used for the assay. The varying volumes (20 µl, 40 µl, and 100 µl) of tissue extract were used for DPPH scavenging. The amount of tissue required (in terms of mg of protein) for scavenging 50% DPPH was measured at 517 nm on BioTek Spectrophotometer.

2.8. Lipid Peroxidation

Lipid peroxidation was measured by thiobarbeturic acid (TBA) reactive substances assay [17,18]. The whole body extracts of adults (15 males and 15 females) homogenized in KCl and were made to react with TBA reagent (containing 0.037 g TBA, 15% TCA and 0.24 N HCl per 10 ml). Reaction mixtures of samples as well as different concentrations of standard malondialdehyde (MDA) were boiled for 15 min, and the coloured product was measured at 532 nm on BioTek spectrophotometer. The concentration of MDA in the samples was estimated using the standard MDA graph plot and represented as µM/mg of protein.

2.9. Reverse Transcriptase-PCR and Real-time Quantitative PCR (RT-qPCR)

The total cellular RNA was extracted using TRIZOL method as per manufacturer's instruction (Merck). Subsequently the cDNA was

prepared using SD-prodigy cDNA synthesis kit (SDFine Chem Ltd.). Primers for actin, cap "n" collar C (cncC), heat shock protein 70 (hsp70) and catalase [Table 1] were designed using the NCBI primer design tool.

Amplification of these genes was carried out with the help of PCR using the reaction setting as 94°C for 2 min, repeated cycle of (25 cycles): Each of 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s with final extension at 72°C for 5 min. The products thus obtained were then run on 2% agarose gel. The gel was observed under ultraviolet transilluminator and the images were captured in Uvitech system. The semi-quantitative estimation of changes in gene expression was carried out in the captured gel images using "ImageJ" software. Following this RT-qPCR was carried out using syber-based EVAGREEN (Biorad) on CFX96 Real-time System (Biorad). The cT values were analyzed for fold change in expression of genes using actin as an internal control. Data from both the experiments were compiled and any mismatch data were repeated for RT-qPCR to obtain accurate results (triplicates of real-time PCR were not performed due to paucity of funds).

2.10. Statistical Analysis

All the experiments were performed in minimum of three sets, except for RT-qPCR. Statistical significance between two mean values was analyzed using Student's *t*-test. For comparing more than two means, analysis of variance (ANOVA) test was performed. ANOVA was subsequently followed by Bonferroni and Holm multiple comparison *post hoc* analysis. For comparing other intergroup differences (between test groups) Tukey's honest significant difference (HSD) *post hoc* method was performed. Throughout the manuscript, * and ** represents Holm inferences $P < 0.05$ and $P < 0.01$, respectively; while # and ## represents Tukey HSD inference $P < 0.05$ and $P < 0.01$, respectively.

3. RESULTS AND DISCUSSION

3.1. Effect of Heat Stress on the Catalytic Antioxidant Mechanism

The flies from different feeding regimes exposed to high temperature exhibited statistically significant variations in the activity of the SOD enzyme (ANOVA *P*: 0.000414). The control flies, exposed to high temperature, showed about 40% increase in the level of SOD activity as compared to control flies at ambient temperature [Figure 1a and Table 2]. The percent increase in the activity of the SOD enzyme in the flies from *Abhrak bhasma* and ascorbic acid feeding regimes and exposed to heat was significantly less than the control flies (Holm inference: $P < 0.01$, $P < 0.05$, and $P < 0.01$ for 0.1% AB, 0.5% AB, and ascorbic acid, respectively). The percent change in the activity of SOD enzyme between flies from 0.1% AB and 0.5% AB feeding regimes also differed significantly (Tukey's HSD inference: $P < 0.05$). The flies from 0.5% AB feeding regime exhibited negative response in activity of SOD enzyme under heat stress.

The flies from all feeding regimes showed an apparent increase of about 12–20% in the activity levels of catalase due to heat stress [Figure 1b and Table 2]. The percent change in catalase activity between flies under different feeding regimes and exposed to heat, however, was found to be statistically insignificant (ANOVA *P*: 0.5417).

3.2. Modulation of Non-catalytic Parameters in Heat Stress

The early chemical response to any stress condition is mainly mediated through non-catalytic antioxidant mechanism. Among these, the glutathione system plays a key role and can be monitored in terms of total GSH content and GSH:GSSG ratio. The control flies showed an apparent decrease in the total GSH content in response to the heat stress, whereas flies from *Abhrak bhasma* and ascorbic acid feeding regimes exhibited an apparent increase in the total GSH content after exposure to high temperature [Figure 2a and Table 2]. However, due to heat stress, only the flies fed with 0.1% AB exhibited a significant percent increase in the total GSH content in comparison to the control flies (ANOVA *P*: 0.013, Holm inference: $P < 0.01$).

In contrast, the GSH: GSSG ratio varied significantly in response to heat stress in flies under different feeding regimes (ANOVA *P*: $1.22*10^{-6}$). The flies from control and 0.5% AB feeding regimes exhibited about 70% increase in the GSH: GSSG ratio in response to heat stress [Figure 2b and Table 2]. The flies from 0.1% AB and ASC feeding regimes, on the contrary, showed a decrease in the GSH: GSSG ratio due to heat stress. The percent change in the GSH: GSSG ratio of

Table 1: Genes and primers used.

Genes	Product size	Forward	Reverse
Actin	469 bp	CGGCTCGGACAGTGATAGAC	CCGGTACCAAGTATCCTCGC
cncC	147 bp	AGCGCTAGGCTAAAGCAACA	GACAGTTAACGGGACGCTCT
Hsp70	188 bp	TTGACAACCGGCTAGTCACT	GGTGTAGAAGTCTTGGCCCT
Catalase	366 bp	GAACTGCCCGTACAAGGTGA	GTCAGCATGCGACCGAAATC

cncC: Cap "n" collar C, Hsp70: Heat shock protein 70

Table 2: Percent change in various oxidative parameter due to heat stress.

Parameters studied	% Change			
	Control	ASC	0.1% AB	0.5% AB
SOD activity	40.6±8.8	−5.1±10.5	13.4±3.4	−25.2±6.1
Catalase activity	12.6±5.5	16.3±3.6	21.6±7.3	12.3±2.6
DPPH scavenging activity	2.6±4.0	−21.0±7.3	−20.7±3.1	−6.9±1.9
MDA content	−5.6±6.9	34.0±6.0	2.3±5.5	5.8±7.8
Total GSH	−14.2±14.0	24.1±6.9	52.0±13.5	14.9±1.2
GSH:GSSG ratio	72.7±6.1	−14.3±4.8	−28.4±9.0	67.6±11.4

Numbers following "±" represents standard error values. SOD: Superoxide dismutase, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, MDA: Malondialdehyde, GSH: Reduced glutathione

flies from 0.1% AB and ascorbic acid feeding regime was significantly lower in comparison to the control flies (Holm inference: $P < 0.01$ and $P < 0.01$ respectively). The percent decrease in GSH: GSSG ratio in these flies was also statistically significant in comparison to the response of flies from 0.5% AB feeding regime (Tukey HSD inference: $P < 0.01$ for 0.1% AB as well as ASC feeding regimes).

The capacity of an organism to scavenge free radicals is yet another simple indicator of antioxidant capacity. It also provides a good estimation of the ability of an organism to tackle the oxidative stress. This was performed *in vitro* using DPPH as the synthetic source of free radicals. The increase in amount of tissue (measured in terms of amount of protein in tissue extract) required for scavenging 50% of the DPPH radicals, represents proportionately lower free-radical scavenging capacity of the tissue. The control flies showed slight decrease in free-radical scavenging capacity under heat stress in comparison to control flies not subjected to heat treatment [Figure 3a and Table 2]. The flies from *Abhrak bhasma* and ascorbic acid feeding regimes, experiencing exposure to high temperature, exhibited an apparent increase in the free-radical scavenging capacity. The flies from 0.1% AB as well as ascorbic acid feeding regimes exposed to heat stress exhibited a significant increase in DPPH scavenging capacity compared to control flies (ANOVA P: 0.001, Holm inference: $P < 0.01$ for both). The standard antioxidant, ascorbic acid, had a response to heat treatment that was not much different than that in flies from 0.1% AB feeding regime.

3.3. Heat Stress Effect on Lipid Peroxidation

The exposure of flies to heat for 1 h did not produce any significant increase in the MDA levels in flies from control as well as *Abhrak bhasma* feeding regimes [Figure 3b and Table 2]. On the contrary, flies from ascorbic acid feeding regime exhibited about 30% increase in the MDA levels. The percent increase in the lipid peroxidation of flies

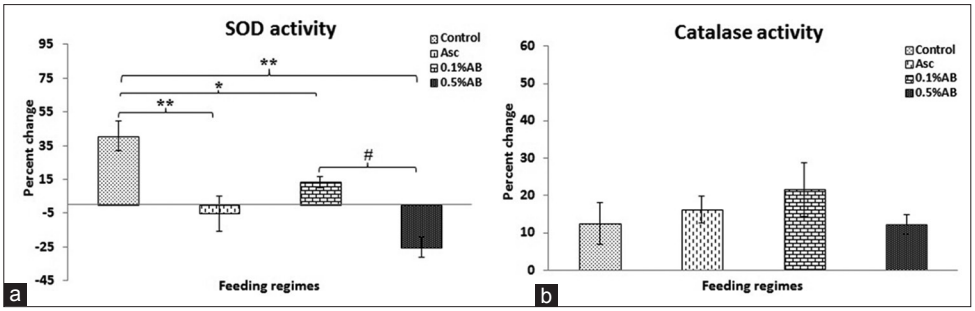

Figure 1: Effect of heat stress on catalytic components. (a) Superoxide dismutase (SOD) activity. (b) Catalase activity. Percent change in activity of SOD and catalase enzymes in flies from different feeding regimes, exposed to heat stress, has been shown in relation to their respective feeding controls ("*" and "**" represents Holm inferences $P < 0.05$ and $P < 0.01$, respectively; while "#" represents Tukey's honest significant difference inference $P < 0.05$).

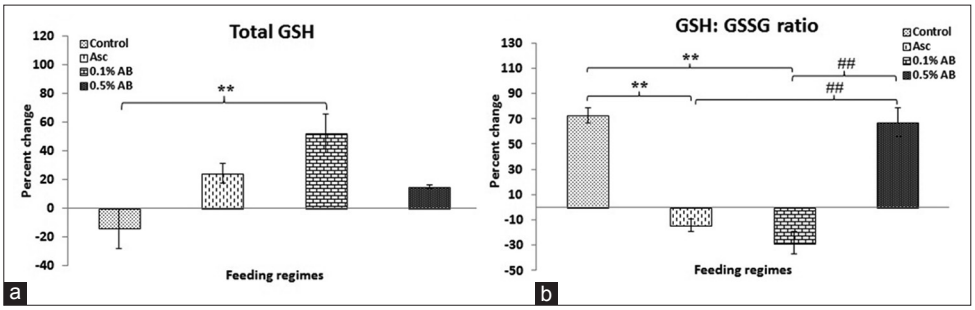

Figure 2: Effect of heat stress on non-catalytic components. (a) Total reduced glutathione (GSH) content. (b) GSH: GSSG ratio. Percent change in total GSH content and GSH: GSSG ratio in flies from different feeding regimes, exposed to heat stress, has been shown in relation to their respective feeding controls ("**" represents Holm inferences $P < 0.01$, while "##" represents Tukey's honest significant difference inference of $P < 0.01$).

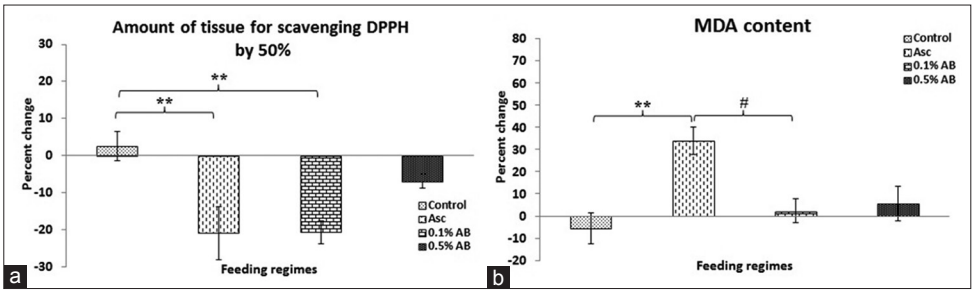

Figure 3: Effect of heat stress on parameters indicative of oxidative stress. (a) Free-radical scavenging capacity (higher the amount of tissue (protein) required for scavenging 50% of 2, 2-diphenyl-1-picrylhydrazyl lower will be the free-radical scavenging capacity). (b) Extent of lipid peroxidation (measured as malondialdehyde content in tissue).

from ascorbic acid feeding regime was significantly higher than the flies from control (Holm inference: $P < 0.01$) as well as 0.1% feeding regime (Tukey HSD inference: $P < 0.05$).

3.4. Changes in the Gene Expressions Due to Heat Stress

The flies from each feeding regime showed variations in the molecular expression of cncC, hsp70, and catalase genes in response to heat stress [Figure 4]. The flies from control and ascorbic acid feeding regimes showed no apparent change in the expression of cncC after exposure to heat stress. The flies from *Abhrak bhasma* (0.1% as well as 0.5% concentration) feeding regimes, on the contrary, exhibited an increase by >1.5 fold in expression of cncC gene, in response to heat stress.

A similar pattern of response in expression was observed for the hsp70 and catalase genes. Only the flies from AB feeding regime showed a distinct increase in the molecular expression of hsp70 as well as catalase gene due to heat stress. The flies from 0.1% AB feeding regime, showed an increase in the expression of hsp70 gene by >3.5 fold while flies from 0.5% AB registered an increase of 2 fold in response to heat stress. Whereas, flies from both 0.1% as well as 0.5% AB feeding regimes exhibited about 1.5-2-fold increase in expression of catalase gene. The flies from control and ascorbic acid feeding regimes showed no apparent change in the expression of these genes after exposure to heat for 1 h. However, only a slight increase in the expression of catalase gene was observed for the flies from control feeding regime in response to heat stress.

Increase in the global temperature is causing a distinct alteration in the expectancy of various organisms since heat stress has been shown to cause changes at physiological as well as molecular levels [2]. These effects are more pronounced in poikilothermic animals as they are incapable of regulating their metabolism independently of the external environment. The physiological changes due to heat stress reflect in the alterations in the parameters indicative of oxidative stress [3]. The current study has been carried out to comprehend these alterations in the oxidative parameters in insects using *Drosophila* as a model system. The investigation is also focused on evaluating efficacy of the dietary supplements of AB and ASC as artificial and natural antioxidant compounds, respectively, in ameliorating the effects of heat stress. Various catalytic, non-catalytic, and molecular parameters have been studied to understand the effects of stress in some details.

The SOD and catalase enzymes are among the most prominently affected components, of the catalytic antioxidant machinery, during the induction of oxidative stress. These enzymes also have an influence on aging process as well [19]. Heat stress has been found to increase activity of the SOD enzyme in control flies. Fleming *et al.* (1994) have reported a similar increase in the activity as well as at expression level of SOD in heat-stressed *Drosophila* flies [20]. The flies from *Abhrak bhasma* feeding regimes have shown relatively lesser increase in activity of the SOD enzyme in response to the heat stress. Flies from the ascorbic acid feeding regime, however, have not exhibited any significant change in activity of the SOD enzyme. These results, thus, indicate that heat stress induces ROS and/or free-radical generation and to manage these radicals activity of SOD enzyme increases. The supplementation of diet with *Abhrak bhasma*, as artificial antioxidant and ascorbic acid, as natural antioxidant, does not seem to bring about noticeable change in the SOD activity.

On the contrary, flies from all feeding regimes have shown an increase in the activity of catalase enzyme. However, the percent change in the activity of catalase in these flies after exposure to heat does not differ significantly, from one group to another. At molecular level, the flies from different feeding regimes have shown differences in the catalase gene expression. Supplementing the diet with *Abhrak bhasma* at 0.1% and 0.5% concentration seems to have caused a response in catalase gene expression contrasting the one in activity of catalase in response to heat. Iron is one of the major constituents of *Abhrak bhasma* and various derivatives of iron have been shown to interact with free radicals, as well as hydrogen peroxide [21]. Therefore, *Abhrak bhasma*, particularly when consumed in higher quantity, by itself can take over

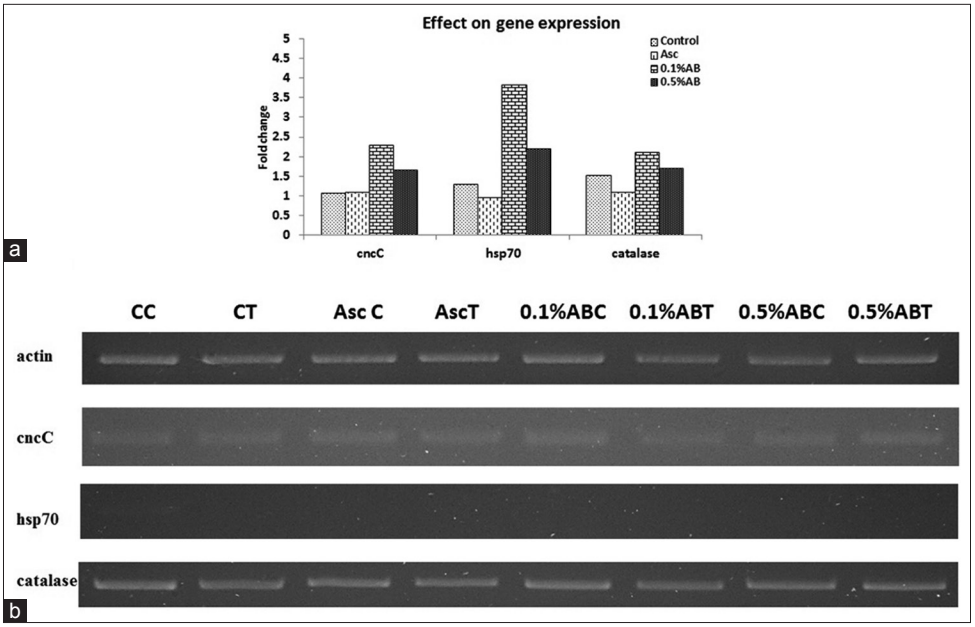

Figure 4: Influence of heat stress on expression of stress-related genes. (a) Real-time polymerase chain reaction (PCR) result of flies exposed to heat stress under various feeding regime. (b) Semi-quantitative result of reverse transcriptase-PCR (PCR products run on 2% gel. These gels were further analyzed using ImageJ software for differences in expression levels.

the function of catalytic components. Hence, supplementation of diet with these compounds directly or indirectly might be participating in reduction of the free radicals being produced in the cellular system in response to heat stress.

The control flies have exhibited an apparent decrease in the total GSH content in response to heat stress though not statistically significant. This might be due to the moderate utilization of GSH in reducing the ROS and/or free radicals. The flies from ascorbic acid feeding regimes have shown an increase in the total GSH content probably since ascorbic acid itself is a reducing agent and might, therefore, donate electrons to the ROS/free radicals in place of GSH, reducing the load on it and resulting in its surplus [22]. However, in flies from *Abhrak bhasma* feeding regimes also, there has been a greater GSH content in comparison to control flies suggestive of the fact that the *bhasma* can induce cellular system to generate reducing potential. The GSH:GSSG ratio, on the contrary, has been noticed to have increased in the flies from control regime perhaps due to efficient enzymatic reduction of GSSG to GSH, which might not have been influenced much by heat. A similar but rather little more remarkable increase in GSH: GSSG ratio has been observed in flies from 0.5% AB feeding regime after exposure to heat. This endorses the capacity of *Abhrak bhasma* in inducing acquisition of reducing capacity by the system in a concentration-dependent manner. The flies provided with diet supplemented with ascorbic acid show a somewhat decrease in the GSH: GSSG ratio probably because the dependence on GSH is reduced and a possible consumption of GSH in some other metabolic pathways [23].

Similarly, alterations in the capacity of tissue to scavenge free radicals can indicate changes induced in the catalytic as well as non-catalytic components in response to heat. In this regard, flies from *Abhrak bhasma* at 0.1% concentration and ascorbic acid feeding regimes have shown an increase in the general free-radical scavenging capacity in response to heat stress. At higher concentration (0.5%), *Abhrak bhasma* itself might be adding to free-radical generation through Fenton-like reaction, due to its high iron content [21].

Exposing flies to heat stress for 1 h does not produce significant increase in the extent of lipid peroxidation in flies from control as well as *Abhrak bhasma* feeding regimes. However, ascorbic acid feeding seems to trigger the lipid peroxidation in the tissue under heat stress. Sestini *et al.* (1991) have reported a similar effect of ascorbic acid, which has increased lipid peroxidation resulting in reduced lifespan of *Drosophila* [24]. The exact mechanism of this response, however, remains elusive.

The cncC and hsp70 gene play a key regulatory role throughout the lifespan of an organism. The expression of cncC gene generally declines with age. However, during oxidative stress, the expression of cncC as well as hsp70 gene increases to rescue cells from such situations [25,26]. In flies from ascorbic acid feeding regime, subjected to heat treatment, the expression of these genes has not been altered. This suggests that ascorbic acid is not very effective in extending protection against heat stress, at least at molecular level. In comparison, flies from control feeding regime show moderate increase in the hsp 70 gene only. In this regard, supplementation of *Abhrak bhasma* at 0.1% as well as 0.5% concentration induces a larger increase in the expression of cncC as well as hsp70 genes. Thus, it additionally strengthens the capacity of cells to counter the effect of heat stress [27].

4. CONCLUSION

Heat stress seems to produce changes in various oxidative parameters in *D. melanogaster.* The diet supplementation of *Abhrak bhasma* specifically at 0.1% concentration helps to cope up with heat stress quite efficiently. Ascorbic acid and 0.5% *Abhrak bhasma* also seem to boost the antioxidant parameters in *Drosophila* flies; however, they also have slightly negative effect on a few of the antioxidant parameters.

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