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Effect of Antioxidant Rich Spices, Clove and Cardamom Extracts on the Metabolic Enzyme Activity of *Labeo rohita*

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Abstract

The present study was conducted to evaluate the protective effect of clove (Cl) and cardamom (Cd) extracts as natural antioxidants in the diet of *Labeo rohita* fingerlings. Both the spice extracts were mixed separately or in combination at the level of 0.5 and 1.0%. Thus, seven experimental diets were Cl-0.5, Cl-1.0, Cd-0.5, Cd-1.0, CC-0.5 and CC-1.0 and control with no extract. The results revealed that, SOD and CAT activities were significantly ($p<0.05$) higher in Cl-0.5 compared to the control. AST, ALT, LDH, MDH, G-6-Pase, activities in liver and muscle decreased significantly ($p<0.05$) in all treated groups. Significantly ($p<0.01$) lowest activity was observed in treatment group fed with clove extract (0.5%) as compared to the control. The results suggest that the dietary administration of clove extract supplementation at a concentration of 0.5% level possess good anti-stress activity.

Keywords: Clove; Cardamom; *Labeo rohita*; Antioxidant

Introduction

When stress becomes extreme, it is harmful for the body, and hence needs to be treated. Stress is involved in the pathogenesis of a variety of diseases that include immunosuppression, endocrine disorders etc. The response to stress in fish is characterized by the stimulation of the hypothalamus, which results in the activation of the neuroendocrine system and a subsequent cascade of metabolic and physiological changes [1,2]. These changes enhance the tolerance of an organism to face an environmental variation or an adverse situation while maintaining a homeostasis status [3,4]. Stress is a common phenomenon that is experienced by every organism.

Stress causes immune-suppression leading fish vulnerable to different diseases. Use of antibiotics to treat such diseases has negative effects on animal's production such as residues in tissues, withdrawal period, and development of resistance to microorganisms [5]. As an alternative, herbs, spices, and various plant extracts have received increased attention as possible replacement of antibiotics**.** In this view, aromatic plants and essential oils extracted from these plants became interesting due to their antimicrobial [6], antioxidant [7] effects and their stimulating effects on animal performance [8,9] and digestive enzymes [10].

Eugenia cryophyllus (clove), belongs to the family Myrtaceae, has a number of medicinal properties and its systemic and local use has been advocated in traditional medicine. Clove is reported to possess anti-oxidant [11], anti-pyretic [12], anti-candidal [13], antibacterial [14], local anesthetic [15], and aphrodisiac [16] activities. It is widely used as an aromatic stimulant, antispasmodic and carminative spice. Clove contains 14-20% of volatile oil that includes eugenol, acetyleugenol, sesquiterpenes (α- and β-caryophyllenes) and small quantities of esters, ketones and alcohol. Clove also contains tannins, sitosterol and stigmosterol [17].

Cardamom (Elettaria cardamomum) belongs to family zingiberaecae, is a sweet spice and is employed as a medicinal flavoring agent, and it has been reported to possess antioxidant, anti-inflammatory, for indigestion, appetite stimulant, carminative and it has positive effects when used as feed additive for poultry [18-21]. Antimicrobial and essential oil content [22-24]. Since clove/cardamom has a number of medicinal properties and is a potent anti-oxidant, the present study was undertaken to evaluate their anti-stress effect in *Labeo rohita*.

Materials and Methods

Fish and culture conditions

Labeo rohita (average weight 15.05 ± 2.1 g) were obtained from Mahaad Fish Farm, Maharashtra, India and transported to wet lab of Central Institute of Fisheries Education, Mumbai, India and acclimatized for 15 days in 500- L fiber glass tanks. During the experimental period, 50% of water was renewed daily to maintain the water quality. Feeding was done twice daily to satiation. Mean water temperature, pH and dissolved oxygen were around 25 ± 2.0°C, 7.6 and 6.6 ± 0.01 ppm, respectively during the experimental period.

Spice extracts

The extracts were prepared according to the method previously described with some modification [25]. The spices were ground into powder in a laboratory grinder and sieved into fine powder to be used for extraction. About 10 g of finely powdered clove and cardamom was weighed separately and extracted with ethyl acetate in a soxhlet's apparatus for at least 24 hrs at 70°C. The solvent with extract was filtered with Wathman no.1 filter paper and centrifuged at 5000 rpm for 5 min to obtain clear supernatant. In order to get pure extract, the solvent was removed by using a rotary evaporator (IKA HB 10 basic, Labortechnik, Saufen, Germany) at 70°C. Solvent fee extract was finally stored at 4°C until use.

Experimental design and diets

Seven experimental diets were formulated (Table 1), so as to contain respective concentrations of clove and cardamom extracts separately and in combination. The diets contained extracts of clove and cardamom, control (0%), Clove extract (Cl-0.5% and 1.0%),

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Treatments	Gill	liver	Serum
Control	$50.05^{e} \pm 0.63$	$44.67^{d} \pm 1.50$	$14.13^{d} \pm 1.08$
Clo-0.5	$21.67^{a} \pm 1.04$	$17.83^{a} \pm 1.51$	$3.10^{a} \pm 0.16$
Clo-1.0	$34.23^{b} \pm 2.31$	$31.04^{b} \pm 1.09$	$4.64^{b} \pm 0.36$
Cdm-0.5	$34.86^{bc} \pm 2.97$	$39.94^{c} \pm 0.79$	$5.71^{b} \pm 0.44$
Cdm-1.0	$42.28^{d} \pm 0.44$	$39.80^{c} \pm 0.47$	$11.82^{c} \pm 0.26$
CC-0.5	$39.97^{cd} \pm 0.32$	$34.61^{c} \pm 1.07$	$6.12^{b} \pm 0.69$
CC-1.0	$44.78^{d} \pm 2.22$	$40.26^{c} \pm 1.74$	$6.17^{b} \pm 0.61$
P value	0.000	0.000	0.000

Table 1: Superoxide dismutase (SOD) in liver, gill and serum of different experimental groups.

Mean values bearing different superscripts under each column vary significantly ($P<0.05$).

Data expressed as mean ± SE, n=3.

SOD specific activity is expressed in 50% inhibition of epinephrine auto oxidation/mg protein/min.

Cardamom extract (Cd-0.5% and 1.0%), Clove+Cardamom extract [(C.C- 0.5% (1:1)] and Clove+Cardamom extract [1.0% (1:1)]. All the ingredients were pressed through a pelletizer (1 mm dia). The pellets were dried at room temperature for overnight and stored at -20°C until use. Feed were given to satiation twice a day throughout the eight weeks of feeding trial. Three hundred fifteen fish (n=315) were equally divided into seven treatment in triplicate ($7 \times 3=21$), where 15 fish were stocked in (50 L capacity) tanks.

Tissue homogenate preparation

The muscle, liver and gill of the fishes were removed carefully and were weighed. It was homogenized with chilled sucrose solution (0.25 M) in a glass tube using tissue homogenizer. The tube was continuously kept in ice to avoid heating. The homogenate was centrifuged at 5000 rpm for 10 minutes at 4°C in a cooling centrifuge machine. The supernatant was stored at 4°C till the use. A 5% homogenate was prepared for muscle, liver and gill.

Plasma superoxide dismutase (SOD)

SOD activity was assayed according to the earlier method [26], which is based on the oxidation of epinephrine to adrenochrome by the enzyme. 0.1 ml of tissue homogenate was added to the tubes containing 0.75 ml of ethanol and 0.15 ml of chloroform (chilled in ice) and centrifuged. 0.5 ml of EDTA solution and 1 ml of buffer were added to 0.5 ml of supernatant. The reaction was initiated by the addition of 0.5 ml of epinephrine and the increase in absorbance (480 nm) was monitored at 30 sec for 3 min. Enzyme activity was expressed as 50% inhibition of epinephrine auto-oxidation/min/mg protein.

Catalase (CAT)

Catalase activity was assayed according to the method described earlier [27]. To a reaction mixture of 2.45 ml phosphate buffer (50 mM, pH 7.0), enzyme source was added and the reaction was started by the addition of 1.0 ml of H_2O_2 solution. The decrease in absorbance was measured at 240 nm at 15 sec intervals for 3 min. The enzyme blank was run simultaneously with 1.0 ml distilled water instead of H_2O_2 solution. Enzyme activity was expressed as nano moles H_2O_2 decomposed/min/mg protein.

Lactate dehydrogenase (LDH)

The LDH activity was assayed in different tissues by the method of Wroblewski and Ladue [28]. The total 3 ml of the reaction mixture comprised of 2.7 ml of 0.1 M phosphate buffer (pH 7.5), 0.1 ml of NADH solution (2 mg NADH dissolved in 1 ml of phosphate buffer solution), 0.1 ml of tissue homogenate and 0.1 ml of sodium pyruvate. The reaction was started after addition of substrate sodium pyruvate. The OD was recorded at 340 nm at 30 seconds interval. The enzymatic activity was expressed as units/mg protein/min at 25°C where 1 unit was equal to Δ0.01 OD/min

Malate dehydrogenage (MDH)

The MDH activity was assayed in different tissues by the method of Ochoa [29]. Total 3 ml of the reaction mixture comprised of 2.7 ml of 0.1 M phosphate buffer (pH 7.5), 0.1 ml of NADH solution (2 mg NADH dissolved in 1 ml of phosphate buffer solution), 0.1 ml of tissue homogenate and 0.1 ml of freshly prepared oxaloacetate solution (2 mg oxaloacetate dissolved in 2 ml chilled distilled water). The reaction was started after addition of oxaloacetate solution as substrate. The OD was recorded at 340 nm at 30 seconds interval for 3 minutes. The enzymatic activity was expressed as units/mg protein/min at 25°C where 1 unit was equal to Δ0.01 OD/min.

Glucose 6 Phosphatase (G6Pase)

The G6Pase activity in the tissue was assayed by the method of Marjorie [30]. The assay mixture consisted of 0.3 ml of malate buffer (pH 6.5), 0.1 ml of 0.1 M glucose 6-phosphate solutions and 0.1 ml of tissue homogenate and was incubated for 15 min at 37°C. The reaction was terminated by addition of 1 ml of 10% TCA solution. 1 ml of the aliquot of the supernatant was used for phosphate (Pi) estimation by method of Fiske and Subbarow [31].

Glucose-6-phosphate dehydrogenase (G6PDH)

The G6PDH activity in different tissues was assayed by the method of De Moss [32]. The total 3 ml of the reaction mixture comprised of 1.5 ml of 0.1 M Tris buffer (pH 7.8), 0.2 ml of 2.7 mM NADP, 0.1 ml of tissue homogenate, 1.05 ml of distilled water and 0.1 ml of 0.02 M glucose-6-phosphate (G6P). The reaction was started by adding G6P as substrate. The OD was recorded at 340 nm 15 seconds interval against distilled water. The G6PDH activity was expressed as units/mg protein/minute. One unit was equal to Δ0.01 OD/min/ml at 25°C.

Alanine amino transferase (ALT)

The ALT activity was assayed in the tissue homogenate as described by Wooten [33]. The substrate comprised of 0.2 M, L-alanine and 2 mM α-ketoglutarate in 0.05 M phosphate buffer (pH 7.4). To 0.5 ml of substrate 0.1 ml of tissue homogenate was added and incubated at 37°C for 1 hr. The reaction was terminated by the addition of 0.5 ml of 1 mM 2, 4-dinitrophenylhydrazine (DNPH). In the control tubes, the enzyme source was added after DNPH solution. The tubes were held at room temperature for 20 min. Then 5 ml of 0.4 N NaOH solution was added and the contents were thoroughly mixed. After 10 min. the OD was recorded at 540 nm against blank. Enzyme activity was expressed as n mol of sodium pyruvate releawed/min/mg protein at 37°C.

Aspartate amino transferase (AST)

The AST activity was assayed in different tissue homogenates as described by Wooten [33]. The substrate comprised of 0.2 M D, L-aspartic acid and 2 mM α-ketoglutarate in 0.05 M phosphate buffer (pH 7.4). In the experimental and control tubes, 0.5 ml of substrate was added. The reaction was started by adding 0.1 ml of tissue homogenate. The assay mixture was incubated at 37°C for 60 minutes. The reaction was terminated by adding 0.5 ml of 1 mM 2,4 dinitrophenyl hydrazine (DNPH). In the control tubes the enzyme source was added after DNPH solution. The tubes were held at room temperature for 20 minutes with occasional shaking. Then 5 ml of 0.4 ml NaOH solution

was added, the contents were thoroughly mixed. After 10 minutes, the OD was recorded at 540 nm against blank.

Protein estimation

Total protein of each tissue sample was estimated by Bradford method [34]. Aliquots (20 µl) of the sample were taken in a dry test tubes and the volume was raised to 1 ml by adding distilled water. To this was added 250 µl of NaOH and 5 ml of Bradford reagent (100 mg coommassie blue G250 I 50 ml 95% ethanol mixed with 85% phosphoric acid and made to 1 L). The content were mixed on a cylomixer and allowed to stand for 5 minutes. The absorbance was taken at 595 nm and plotted onto the standard curve to obtain the total protein content of the tissue sample [34]. The standard curve was made using bovine serum albumin (BSA) as the standard.

Statistical analysis

The data were expressed as average mean ± standard error (SE). Statistical analysis of data was done by one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT). The levels of significance were expressed at 5% (P<0.05).

Results

Antioxidant activity

SOD activity of liver was higher than the gill. The highest activity was observed in the control group and the lowest activity was observed in Cl-0.5 group both in liver, gill and serum (Table 1). Similarly, highest catalase activity was observed in the control group and the lowest activity was observed in Cl-0.5 group.

Lactate dehydrogenase (LDH) and Malate dehydrogenage (MDH)

In muscle highest activity (p<0.05) was observed in the control group and the lowest activity was recorded in cl-0.5 g. In liver highest activity (P<0.001) was found in the control group and the lowest activity was recorded in cl-0.5 g group. There was no significant difference between Cl-1.0 g, Cd-0.5 g, Cd-1.0 g, CC-0.5 g and CC-1.0 g (Table 2). In the muscle the highest MDH activity (P<+0.001) was observed in the control group and the lowest activity was observed in Cl (0.5%) group. In liver the highest MDH activity was observed in the control group and the lowest activity was observed in Cl (0.5%) group (Table 3).

Glucose 6 phosphatase (G6Pase) and Glucose-6-phosphate dehydrogenase (G6PDH)

Highest G-6-Pase value of liver was recorded in the control group and the lowest value was recorded in Cl-0.5. There was no significant difference between Cl-1.0, Cd-0.5 and Cd-1.0 (Table 4). Highest activity of G-6-PDH was recorded in Cl-0.5 which is significantly different from all the other groups. The lowest activity was recorded in the control group (Table 5).

Treatments	Gill	Liver	Serum
Control	$5.01^{b} \pm 0.14$	$6.94^{c} \pm 0.22$	$11.74^{d} \pm 0.47$
Clo-0.5	$1.27^{a} \pm 0.13$	$1.93^{a} \pm 0.03$	$6.90^{a} \pm 0.20$
Clo-1.0	$3.32^{b} \pm 0.55$	$3.43^{ab} \pm 0.33$	$8.66^{b} \pm 0.31$
Cdm-0.5	$4.26^{b} \pm 0.94$	$4.46^{abc} \pm 0.70$	$9.72^{bc} \pm 0.43$
Cdm-1.0	$4.03^{b} \pm 0.81$	$3.48^{ab} \pm 0.24$	$10.54^{cd} \pm 0.82$
CC-0.5	$4.14^{b} \pm 0.46$	$2.99^{ab} \pm 0.14$	$10.97^{cd} \pm 0.22$
CC-1.0	$4.23^{b} \pm 0.23$	$5.08^{bc} \pm 0.18$	$10.89^{cd} \pm 0.21$
P value	0.003	0.000	0.000

Table 2: Catalase activity in liver, gill and serum of different experimental groups.

Mean values bearing different superscripts under each column vary significantly (P<0.05).

Data expressed as mean ± SE, n=3.

Catalase specific activity is expressed in nanomoles H_2O_2 decomposed/min/mg protein.

Treatments	Muscle	Liver
Control	$3.19^{e} \pm 0.12$	$2.44^{d} \pm 0.12$
Clo-0.5	$1.31^{a} \pm 0.07$	$1.18^{b} \pm 0.02$
Clo-1.0	$2.34^{cd} \pm 0.44$	$1.10^{a} \pm 0.06$
Cdm-0.5	$1.61^{b} \pm 0.23$	$1.73^{c} \pm 0.08$
Cdm-1.0	$1.62^{bc} \pm 0.28$	$1.19^{bc} \pm 0.04$
CC-0.5	$1.55^{b} \pm 0.22$	$1.57^{bc} \pm 0.21$
CC-1.0	$2.60^{de} \pm 0.10$	$1.53^{bc} \pm 0.33$
P value	0.002	0.000

Table 3: Lactate dehydrogenase (LDH) activity in muscle and liver of *Labeo rohita* fingerlings fed with different experimental diets.

Data were presented as mean ± SE (n=3).

Values within the same column having different superscripts are significantly different (P<0.05).

LDH: specific activity expressed as Units/mg protein/min at 37°C.

Treatments	Liver	Muscle
Control	$3.97^{d} \pm 0.50$	$4.66^{d} \pm 0.04$
Clo-0.5	$1.34^{a} \pm 0.08$	$1.66^{a} \pm 0.08$
Clo-1.0	$1.77^{b} \pm 0.61$	$1.82^{b} \pm 0.07$
Cdm-0.5	$1.82^{bc} \pm 0.55$	$3.24^{c} \pm 0.30$
Cdm-1.0	$1.56^{b} \pm 0.41$	$2.42^{bc} \pm 0.29$
CC-0.5	$1.38^{bc} \pm 0.17$	$2.98^{c} \pm 0.38$
CC-1.0	$1.62^{b} \pm 0.08$	$2.61^{bc} \pm 0.46$
P value	0.000	0.000

Table 4: Malate dehydrogenase (MDH) activity in muscle and liver of *Labeo rohita* fingerlings fed with different experimental diets.

Data were presented as mean ± SE (n=3).

Values within the same column having different superscripts are significantly different (P<0.05).

MDH: Specific activity expressed as Units/mg protein/min at 37°C

Treatments	G-6-Pase	G-6-PDH
Control	$6.47^{d} \pm 0.51$	$10.21^{d} \pm 0.06$
Clo-0.5	$2.01^{a} \pm 0.48$	$31.12^{a} \pm 0.19$
Clo-1.0	$3.67^{b} \pm 0.57$	$21.11^{b} \pm 0.17$
Cdm-0.5	$4.38^{c} \pm 1.45$	$29.23^{b} \pm 0.08$
Cdm-1.0	$4.45^{c} \pm 1.76$	$25.69^{b} \pm 0.25$
CC-0.5	$4.78^{c} \pm 1.56$	$27.34^{b} \pm 0.16$
CC-1.0	$3.69^{b} \pm 0.59$	$16.81^{c} \pm 0.08$
P value	0.000	0.000

Table 5: Gluose-6-phosphatase (ng phosphorus release/min/mg protein) and Gluose-6-phosphate dehydrogenase (U/mg protein/min) in liver of different experimental groups.

Data were presented as mean ± SE (n=3).

Values within the same column having different superscripts are significantly different (P<0.05).

Alanine amino transferase (ALT) and Aspartate amino transferase (AST)

In the muscle the highest activity of ALT was observed in control group which is significantly different from all other groups and the lowest activity was recorded in Cl-0.5. In the liver the highest activity was observed in control group and the lowest activity was recorded in

Cl-0.5 group (Table 6). The AST enzyme activity in liver and muscle differ significantly (P<0.001). In the muscle the highest activity was observed in control group and the lowest activity was recorded in Cl-0.5 group. In the liver highest value was recorded in control group which is significantly different from other groups and the lowest value was observed in Cl-0.5 group (Table 7).

Discussion

The production of free radicals in the body is countered by antioxidant enzymes like SOD and catalase. The mechanism of their protective function is different: superoxide dismutase mainly catalyzes cell defense reaction against potentially harmful effects of superoxide (O_2^-) produced during metabolism. SOD activity was observed lowest in the Cl-0.5 group, suggesting minimum production of free radicals as much of these free radicals are being scavenged by clove extract at 0.5%, which also showed highest anti-oxidant activities. The antioxidant activity of clove could have attributed to its phytochemical contents for the protective effect [35], or due to their trace element contents which are required for the antioxidant enzyme activity [36]. Nooman et al. reported that serum total lipids decreased by increasing levels of cardamom and this may be due to antioxidant activity of cardamom that decreases lipids per oxidation [37].

Lactate dehydrogenase is markers of skeletal and myocardial muscle [38,39]. When myocardial cell, containing LDH are damaged or destroyed due to deficient oxygen supply or glucose, the cell membrane becomes permeable or may rapture, which results in the leakage of enzymes [40]. In the present study, lactate dehydrogenase (LDH) significantly reduced (p<0.05) in the treatment group fed with clove extract at 0.5% as compared to the control group, suggesting that extract of clove (Syzygium aromaticum) had protective effects on the muscle and liver of *Labeo rohita*. The present results are in agreement with Gabriel [41] who found that LDH activity was reduced in *Clarias gariepinus* when injected with aqueous extracts of *Lepidagathis alopecuroides* leaves. A reduction in the concentration of LDH in the plasma of the experimental fish infers a decrease in the glycolytic process due to lower metabolic rate [42], a shift towards anaerobic respiration [43], possibly due to a hypoxic internal environment.

Treatments	Liver	Muscle
Control	$11.36^{d} \pm 0.21$	$31.65^{e} \pm 0.24$
Clo-0.5	$9.01^{a} \pm 0.24$	$26.55^{a} \pm 0.26$
Clo-1.0	$9.29^{b} \pm 0.42$	$29.16^{bc} \pm 0.50$
Cdm-0.5	$10.98^{c} \pm 0.38$	$30.32^{c} \pm 0.30$
Cdm-1.0	$9.78^{b} \pm 0.29$	$30.78^{cd} \pm 0.38$
CC-0.5	$10.11^{c} \pm 0.37$	$29.27^{d} \pm 0.29$
CC-1.0	$9.32^{b} \pm 0.11$	$28.42^{d} \pm 0.33$
P value	0.000	0.002

Table 6: Aspartate amino transaminase (AST) activity in liver and muscle of different experimental groups.

Mean values bearing different superscripts under each column vary significantly (P<0.05).

Data expressed as mean ± SE, n=3.

AST: Specific activities expressed as nanomoles of oxaloacetate released/min/mg protein at 37°C.

Treatments	Liver	Muscle
Control	$12.31^{e} \pm 0.26$	$30.13^{e} \pm 12.42$
Clo-0.5	$9.21^{a} \pm 0.12$	$27.01^{a} \pm 3.01$
Clo-1.0	$10.32^{bc} \pm 0.24$	$28.38^{b} \pm 10.90$
Cdm-0.5	$11.29^{c} \pm 0.19$	$29.95^{bc} \pm 10.84$
Cdm-1.0	$11.76^{cd} \pm 0.32$	$29.12^{bc} \pm 12.73$
CC-0.5	$10.31^{d} \pm 0.39$	$28.93^{cd} \pm 4.91$
CC-1.0	$9.89^{d} \pm 0.21$	$27.61^{d} \pm 2.44$
P value	0.013	0.001

Table 7: Alanine amino transaminase (ALT) activity in liver and muscle of different experimental groups.

Mean values bearing different superscripts under each column vary significantly (P<0.05).

Data expressed as mean ± SE, n=3.

Malate dehydrogenase (MDH) activity significantly reduced in the treatment group fed with clove extract at 0.5% as compared to the control group. It follows the same trend as that of LDH. The decreased MDH activity level in the present study indicates the decreased operation of Krebs cycle probably by limiting the flow of substrates into the cycle or impairment of mitochondrial organization. The energy demand of fish increases during stress and increase the MDH activity. Padmaja [44] reported that curcumin, a known anti-oxidant, seems to protect the Kreb's cycle enzyme systems possibly mediating through scavenging free radicals that may be generated under selenium stress in wistar rat.

Glucose-6-phosphatase (G-6-Pase) activity in the tissues of *Labeo rohita* significantly reduced in response to treatment with clove extract at 0.5% as compared to the control. With respect to G-6-Pase as glycogenolytic enzyme, it exhibited reduced activity in *Labeo rohita* which was attributed to synthesis and/or degradation of glycogen [45]. Increasing the glucose concentration stimulated glycogen synthesis and decreased the activity of glycogen phosphorylase. Glucose was incorporated into glycogen during period of net glycogen breakdown, and vice versa; glycogen degradation occurred during periods of net glycogen synthesis which depends on glucose concentration [46].

The present results showed a significant increase in G-6-PD activity level in the liver of *Labeo rohita* fed with clove extract at 0.5% in comparison to the control. Succinate dehydrogenase is an important active regulatory enzyme of the tricarboxylic acid cycle (TCA), the common pathway for carbohydrates. While, G-6-PD is the key enzyme that catalyses the oxidative irreversible step of the alternative route of glucose metabolism via hexose monophosphate shunt (HMP). The increase in G-6-PD activity indicated the mobilization of glucose through pathways other than glycolysis-Kerbs' cycle axis and indicates the high rates of pentose-phosphate pathway. In the present study it is suggested that the increased activity of G-6-PD to reflect the differential effects of stress. Hexose monophosphate (HMP) shunt stress conditions have been reported by Gonzalez and Tejedor [47].

Both amino-transferases activity were studied in liver and muscle and there was significant difference of ALT and AST in the treatment group fed with clove extract at 0.5% as compared to the control. Hanumanthappa [48] reported that dietary curcumin, capsaicin and their combination reduced activities of serum enzymes, ALT, AST and LDH, indicating that these spice principles reduce the severity of iron-induced hepatotoxicity by lowering lipid peroxidation. ALT and AST levels increased significantly in *Labeo rohita* infected with A. hydrophila, and elevated levels of ALT and AST were brought back to normal by Achyranthes treatment [49]. The results of the present study are also in agreement with El-Segaey [50], who studied the antioxidant and hepatoprotective effects of clove and cardamom in ethanol induced hepatotoxicity and found that ALT and AST are significantly reduced in treated group as compared to only ethanol treated and control group. As also observed by Abdel-Wahha [51] treatment

with clove and cardamom effectively decreased liver enzyme levels in the serum. This can be attributed to the presence of antioxidant in clove and cardamom which contain phenolic compounds that can act by scavenging free radicals. In addition, clove and cardamom extract may inhibit the biotransformation of ethanol to acetaldehyde and consequently decreases ROS formation and consequently have protective function. The decline in ALT in treated fish in this study may be due to the fall in the rate of synthesis of glycogen resulting from the low metabolic demands [52] and a decrease in metabolic transport [53]. The preventive effect of clove on the stress induced biochemical changes indicating its anti-stress activity. The effect of clove may be due to its effect on the central nervous system or endocrines and it may also be due to its antioxidant effect as antioxidants are known to prevent stress induced damage due to generation of free radicals.

Conclusion

It is known that anti-oxidants can be beneficial for the prevention of stress induced pathological changes. Clove extract may be responsible for prevention of oxidative related diseases due to the presence active compound eugenol. The exact mechanism by which clove produces its anti-stress activity is not fully understood, however, it is understood that the antioxidant activity of clove buds might contribute at least in part to its anti-stress activity.

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