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Antistress potential of acetone extract of *Zingiber officinale* Roscoe on biochemical and oxidative stress parameters in *Labeo calbasu* (Hamilton 1822) fingerlings subjected to acid stress

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ABSTRACT

A 30 days feeding trial was conducted to study the antistress property of acetone extract of *Zingiber officinale* against acid stress in *Labeo calbasu* fingerlings. Fish fingerlings (7.34 ± 0.06 g; average weight ± SE) were randomly distributed into 4 treatment groups, each with 3 replicates and were fed with four diets containing different levels of *Z. officinale* acetone extract (0, 0.05, 0.5 or 5%). The potential antistress ability of *Z. officinale* acetone extract was assessed in terms of production of serum cortisol, serum glucose, serum cholesterol, serum triglycerides, triiodothyronine (T_3), thyroxine (T4), alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), catalase (CAT), superoxide dismutase (SOD) and glutathione S transferase (GST) while they are exposed to acidic water with pH 5.5 for 24 h. Acid stress significantly increased serum cortisol, glucose, cholesterol, T3, T4, ALT, AST and ALP levels and decreased the triglycerides level in all the treatments. *Z. officinale* acetone extract in fish diet at a minimum level of 0.5% concomitantly reduced the stress in *L. calbasu* fingerlings.

Keywords: Acid stress, Biochemical parameters, *Labeo calbasu, Zingiber officinale*

Introduction

The cultivation of fish has improved significantly in India during past few decades and fishes are exposed to a wide variety of environmental stress factors during culture. Fish, like other vertebrates, respond to stressors by eliciting generalised physiological stress responses, characterised by an increase in secretion of stress hormones and consequent changes that help to maintain the animal's normal or homeostatic state (Iwama m *et al*., 1999; Barton, 2002). This generalised response has been considered to be adaptive and represents the natural capacity of the fish to respond to stress. This response includes, for example, increases in plasma cortisol, catecholamines and glucose levels (Barton and Iwama, 1991). Measurement of metabolic enzyme activity can also serve as a valuable stress indicator in organisms like marine invertebrate and fishes, where the accurate determination of metabolic rate is difficult (Dahlhoff, 2004).

pH of the water plays an important role in maintenance of homeostasis in aquatic animals and decreases in pH reported to cause disturbances in acid–base and ion regulation as well as ammonia excretion (Jensen and Brahm, 1995). Disturbances in gas exchange, nitrogenous waste excretion, acid-base and ionic balance due to changes in water pH cause stress in fish, affecting its body physiology and growth (Pickering, 1981; Jeney *et al*., 1992). Fishes can cope with chronic changes in pH within certain ranges; however, acute changes in pH can result in acidosis if it is below 5.5 (Roberts, 2012). Sudden drop of pH lead to severe distress in some species and the fish may try to escape by jumping out of the water which may lead to death. Changes in the pH or acidity of the environment can alter or totally inhibit the enzyme from catalysing a reaction. The carp culture industry is currently the most important sub-sector of fisheries in India and its rapid development has attracted considerable attention in recent years. In situations of high nutrient input, supplementary feed, manures and inorganic fertilizers into the ponds can cause variations in pH diurnally. During the night, carbon dioxide accumulates and pH declines (Boyd and Tucker, 1998) and a change in water pH, either to higher or lower levels, could cause stress in fish and affect its body physiology and growth (Das *et al.,* 2006).

Natural plant products have been reported to promote various antistress responses, growth, appetite stimulation and immunostimulation. Antimicrobial properties are attributed to the active principles in the natural products such as alkaloids, flavanoids, pigments, phenolics, terpenoids, steroids and essential oils (Citarasu *et al*., 2002; Sivaram *et al.,* 2004). Herbal compounds have the ability to inhibit the generation of oxygen anions and to scavenge free radicals. The herbal antioxidant effect has been shown to be similar to that of superoxide dismutase, metal-ion chelators and xanthine oxidase inhibitors.

Ginger (*Zingiber officinale* Roscoe) has been used as a spice for over 2000 years (Bartley and Jacobs, 2000). The

extracts from the rhizome of *Z. officinale* contain polyphenol compounds (6-gingerol and its derivatives), which have high antioxidant activity (Chen *et al*., 1986; Hermann, 1994). When ginger was included in animal diets, it was found that there was considerable increase in the pancreatic and intestinal lipase (Platel and Srinivasan, 2000). The present work was intended to investigate the antistress activity of acetone extract of *Z. officinale* in *Labeo calbasu* fingerlings exposed to acid stress by analysing the levels of stress hormones and selected enzyme activities of different biochemical pathways.

Materials and methods

Collection of plant and preparation of plant extracts

The rhizomes of *Z. officinale* were collected from Versova, Mumbai, India, and transported to Central Institute of Fisheries Education (CIFE), Mumbai. Dr. Rajendra D. Shinde, Associate professor, Department of Botany, St. Xavier's College, Mumbai confirmed the species identity. Fresh rhizomes of *Z. officinale* were cleaned and shade dried. The dried rhizomes were pulverised in an electrical blender and passed through 20 µm mesh sieve. Sieved powder (500 g) was extracted using Soxhlet apparatus at 60 ºC by the method of Peach and Tracey (1956) using acetone as a solvent. The extract was concentrated in a water bath and after complete evaporation of the solvent, the residues of the extract was stored at 4 ºC for further use. The yield of the extract was 6% in terms of dried starting material.

Preparation of herbal diets

Four isocaloric artificial diets were formulated (Sahu *et al.,* 2007) containing either 0%, 0.05%, 0.5% and 5% *Z. officinale* acetone extracts. Ingredient compositions of the experimental diets are presented in Table 1. All the ingredients except vitamin mineral mixture and *Z. officinale* acetone extract were mixed in a plastic bowl to get homogeneous ingredient mixture. The dough was then allowed for conditioning at room temperature for 1 h followed by steam cocking for 20 min in a pressure cooker. The vitamin mineral mixture and *Z. officinale* acetone extract were added after cooling and mixed thoroughly with other ingredients. Pellets were prepared by hand pelletizer having 2 mm die. Finally, the pellets were air dried for 24 h and kept in a hot air oven at 50 ºC till the desired moisture level (10%) was reached. After drying, the pellets were packed in airtight polythene bags, labelled and stored until use.

Proximate analysis of feed and fish tissue

The proximate composition of the experimental diets was determined following the standard methods of AOAC (2000) and is presented in Table 1. The moisture content was determined by drying at 105 ºC to a constant weight. Nitrogen content was estimated by Kjeldahl (2200 Kjeltec Auto distillation; Foss Tecator, Hoganas, Sweden) method and crude protein was calculated by multiplying nitrogen percentage by 6.25. Crude lipid (CL) was measured by solvent extraction method (1045 Soxtec extraction unit, Tecator, Sweden) using diethyl ether (boiling point, 40-60 ºC) as a solvent, and ash content was determined by incinerating the samples in a muffle furnace at 600 ºC for 6 h. Total carbohydrate was estimated by calculating difference*, i.e.,* total carbohydrate %=100-(CP%+ EE%+ Ash%).

Fish and experimental design

Fingerlings of *Labeo calbasu* were brought from Lembucherra fish farm of Tripura, (India) to the aquaculture wet laboratory, Central Institute of Fisheries Education, Mumbai and were acclimatised to the experimental rearing conditions for 15 days. Acid stress model was used for evaluating antistress activity (Zhou *et al.,* 2004). Two hundred and twenty five *L. calbasu* fingerlings (7.34 ± 0.06 g; average weight ± SE) were randomly distributed into 4 treatment groups in triplicates each with 15 fishes (n=15 fish per group) in plastic tanks of 150 l capacity following a completely randomised design (CRD). The experiment lasted for 30 days. Round-the-clock aeration was provided to all the tanks from a compressed air pump and water was exchanged every alternate day. All the fish were fed manually twice a day (0800 and 1800 hrs) with one of the diets containing 0, 0.05, 0.5 or 5% level of acetone extract of *Z. officinale* at the rate of 3% body weight per day (Table 1). The water temperatures of all the treatments were within the range of 26-27 ºC throughout the experimental period. At the end of 30 days, six fish from each replicate (*i.e.,* 18 fish per treatment) were used for the estimation of cholesterol, glucose, triglycerides, cortisol and enzyme activities and the rest nine fish from each replicate (*i.e.,* 27 fish per treatment) were given acid stress by exposing to acidic water for 24 h and again, the same parameters were analysed. Water pH in tanks was reduced from 7.5 to 5.5 using adequate quantity of hydrochloric acid.

Water quality parameters were checked every week following the methods of APHA (1998).

Sampling procedure

The fish were anaesthetised with CIFECALM (50 $\mu l l^{-1}$) (Verma *et al*., 2007), a herbal anaesthetic formulation containing natural alcoholic extracts of *Eugenia caryophyllata* and *Mentha arvensis* (developed by Central Institute of Fisheries Education, Mumbai, India) and blood was collected from the caudal vein within 2 min after capture using a 2-ml syringe with 26-G needle and immediately transferred to Eppendorf tubes. The tubes were allowed to stand in tilted position for 1 h to collect the serum, which was used subsequently for estimation of serum cortisol, glucose, cholesterol, triglycerides, triiodothyronine (T_3), thyroxine (T4), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatise (ALP).

Liver, muscle and gill samples were collected and 5% homogenate was prepared in chilled sucrose solution (0.25 M) in a mechanical tissue homogeniser. The homogenate was centrifuged at 5,000 rpm at 4 ºC for 10 min, supernatant collected and stored at -20 ºC for enzymatic studies.

The fresh tissue homogenate were used immediately for analysis of superoxide dismutase (SOD), catalase (CAT) and glutathion S transferase (GST) enzymes.

Serum biochemical parameters

Cortisol in fish serum was estimated using a validated cortisol ELISA (LDN) MS E-5000 kit (Diagnostic Systems Laboratories, Mumbai, India) and the serum cortisol was

Table 1. Feed formulation of the different experimental diets (kg^{-1}) and proximate composition of diets and animal tissues

Parameters	Experimental diets			
	Control (0%)	T_1 (0.05%)	T_2 (0.5%)	T_3 (5%)
Ingredients (%)				
Groundnut oil cake	400.0	400.0	400.0	400.0
Fish meal	250.0	250.0	250.0	250.0
Rice bran	200.0	199.5	195.0	150.0
Soybean meal	120.0	120.0	120.0	120.0
Vitamin and mineral mix[a]	20.0	20.0	20.0	20.0
Starch	10.0	10.0	10.0	10.0
Z. officinales extract	0.0	0.5	5.0	50.0
Proximate composition of diet (% Drymatter basis)				
Crude protein (CP)	38.26±0.12	38.39±1.45	38.23±1.48	38.57±1.43
Ether extract (EE)	5.80±0.35	5.31±0.56	5.39±0.76	5.07±0.32
Total carbohydrate	45.14±0.76	45.87±0.94	45.99±1.53	46.55±2.02
Total ash	10.80±0.82	10.43±0.01	10.40±0.46	10.09±1.05
Organic matter	89.20±1.23	89.57±1.45	89.60±2.38	89.91±3.45
Biochemical composition of tissue (%)				
Moisture	74.67±0.067	73.62±0.001	73.36±0.004	73.24±0.002
Crude protein	51.86±0.013	52.73±0.002	53.65±0.003	53.30±0.051
Lipid	16.23±0.001	16.67±0.004	17.41±0.041	17.64±0.022
Ash	14.99± 0.13	14.36±0.003	15.16±0.013	15.66±0.032
Total carbohydrates	16.92±0.001	16.24±0.021	13.78±0.003	13.40±0.101

[a]*Vitamin–mineral mix (EmixTM plus) (quantity per 2.5 kg):Vitamin A - 55,00,000 IU; Vitamin D3 - 11,00,000 IU; Vitamin B2 - 2,000 mg; Vitamin E - 750 mg; Vitamin K - 1,000 mg; Vitamin B6 - 1,000 mg; Vitamin B12 - 6 mg; Calciumpanthothenate - 2,500 mg; Niacinamide - 10 g; Cholinechloride - 150 g; Mn - 27,000 mg; Iodine - 1,000 mg; Fe - 7,500 mg; Cu - 2,000 mg; Zn - 5,000 mg; Co - 450 mg; Ca - 500 g; P - 300 g; Se - 50 ppm; L-Lysine - 10 g; DL-methionine - 10 g.*

expressed as µg dl^{-1}. Serum glucose was estimated using glucose kit (Qualigens Fine Chemicals, Mumbai, India) and cholesterol levels using cholesterol kit (CHOD-POD. Liquid) (Merck, Mumbai, India). Serum cholesterol was expressed as mg dl^{-1}. Triglycerides in fish serum was estimated using triglycerides kit (GPO-POD enzymatic-colorometric) (Merck, Mumbai, India) and expressed as mg dl^{-1}. T_3 in fish serum was estimated using T_3 ELISA 2nd generation (LDN) TF E-2300 kit (Diagnostic Systems Laboratories, Mumbai, India), which was expressed in ng ml^{-1}. Serum T_4 analysed using T_4 ELISA 2nd generation (LDN) TF E-2400 kit, was expressed in µg dl^{-1}.

Alanine aminotransferase (ALT) in fish serum was estimated using GPT/ALT (LDH-NADH, Kinetic UV) kit (Merck, Mumbai, India). and was expressed as Ul^{-1}. Aspartate aminotransferase (AST) formerly called glutamate oxaloacetate (GOT) was assayed by same procedure as for ALT except that the substrate used was L-aspartic acid instead of alanine. Serum alkaline phosphatase (ALP) in serum of fish was assayed using alkaline phosphatase (p-Nitrophenilphosphate, Kinetic) kit (Merck, Mumbai, India). ALP in the serum was expressed as Ul^{-1}.

Oxidative stress parameters

The activity of superoxide dismutase (SOD) was assayed with the oxidation of epinephrine–adrenochrome transition and estimated at 480 nm (Misra and Fridovich, 1972). Catalase (CAT) activity was estimated by the method of Takahara *et al*. (1960), using phosphate buffer (50 mM, pH 7.0), the reaction was started by the addition of H_2O_2 solution, and the decrease in absorbance was measured at 240 nm. Glutathione S-transferase (GST) activity towards CDNB (1-chloro-2,4-dinitrobenzene), was determined spectrophotometrically at 340 nm using the method described by Habig *et al*. (1974). The assay was performed at 25 °C using 100 mM potassium phosphate buffer, pH 6.5, with GSH and CDNB (dissolved in ethanol) at a final concentration of 1 mM each. Activity was calculated from the changes in absorbance at 340 nm (ε 340 nm = 9.6 mM^{-1} cm^{-1}).

One unit of GST activity was defined as the amount of enzyme catalysing the conjugation of 1 µmol of CDNB with GSH per min at 25 °C.

Total protein content was analysed from the supernatant (Lowry *et al*., 1951) for calculating enzyme activities. All the colorimetric assays were carried out using UV–VIS spectrophotometer (E-Merck, Germany).

Statistical analyses

All the parameters were analysed using one-way ANOVA with respect to different diets. Post-hoc test was carried out using Duncan's multiple comparison procedures and Student's t test was performed to compare the data on results recorded pre- and post- acid stress. All the statistical analyses were performed using SPSS 16.0 for Windows.

Results and discussion

Water quality parameters

All the physico-chemical parameters of water such as temperature, dissolved oxygen, nitrite-N and nitrate-N were observed to be within the optimum range of requirements for the fish as suggested by many authors. Optimum temperature required for fish growth is 31 ^{o}C (Das *et al*., 2004), which supports the range of temperature, 26-27 ^{o}C observed during the entire experimental period. The dissolved oxygen level in different experimental tubs was recorded to be within the range of 5.6-6.9 mg l^{-1}, which is within the optimum range of 6-7 mg l^{-1} for cyprinids suggested by Huet (1975). Nitrite-N concentration was recorded in the range of 0.001-0.002 mg l^{-1}, which is well within the permissible range for pond aquaculture (Boyd and Tucker, 1998). Nitrate-N level in a productive pond can be within 0.1-5.0 mg l^{-1} (Boyd and Tucker, 1998). In the present study, nitrate-N (0.002-0.038 mg l^{-1}) was below the toxic level and hence did not affect the fish adversely.

Proximate composition of experimental diets

Balanced formulated practical diets containing all the required nutrients in the optimum level were used in the present study. Data pertaining to the proximate composition of different experimental diets are given in Table 1. The crude protein (%) of the diets varied between 38.23-38.57 which is supported by Renukardhyay and Varghese (1986), who suggested that optimum protein requirement of carp range between 30-45%. The lipid content (%) of the diets was between 5.07-5.8 and the total carbohydrate (%) of the diets was found to be between 45.14- 46.55 which is supported by Sahu *et al*. (2007). The ash content (%) estimated was within the range of 10.09-10.80 and the organic matter content (%) of different experimental diets varied between 89.20-89.91which is supported by Misra (2004).

Biochemical composition of the animal tissue

The data on whole body biochemical composition of *L. calbasu* at the end of 30 days of experiment are also summarised in Table 1. Decreased moisture content was seen with increased in lipid in fish, confirming the inverse relationship of moisture and lipids. These results were supported by Ray and Das (1992). Increased accretion of lipid in tissues was evident with low carbohydrate which may be due to increase in concentration of extract in diets (Papaparakeva-Papoutsoglou and Alexis, 1986). In the present study, although some variation was found in the crude protein content and ash, values did not vary significantly ($p>0.05$) among the treatments.

Serum biochemical parameters

Elevations of plasma cortisol and glucose levels were often used as indicators of stress (Barton and Iwama, 1991). Several studies have demonstrated the effects of stressors on concentrations of the corticosteroid cortisol n fish. Plasma concentration of cortisol is dependent on the duration and strength of the stressor (Barton and Iwama, 1991) for which this parameter has been used as an indicator of stress.

Acid stress enhances the plasma cortisol level in rainbow trout (Brown *et al.,* 1990) and greenback flounder (Barnett and Pankhurst, 1998). Increased cortisol elicits compensatory physiological responses that help fish deal with stress (Mommsen *et al.,* 1999). These alterations could be attributed to stimulation of the hypothalamo-pituitary axis and sympathetic nervous system resulting in liberation of catecholamines and glucocorticoids (Prabhakaran *et al.,* 2003). Both catecholamines and corticosteroids have a lipolytic effect on the adipose tissue, stimulating fat mobilisation and increasing circulating free fatty acids (Mungantiwar *et al*., 1997) which probably results in an increase in plasma cholesterol in response to stress. In the present study, control group registered significantly higher ($p<0.05$) serum cortisol level than the other groups fed with *Z. officinale* acetone extract both before and after acid stress (Fig. 1a) indicating secretion of cortisol due to acid stress. Decreases in serum cortisol accompanied increased levels of *Z. officinale* acetone extract (before and after acid stress), showing that *Z. officinale* acetone extract has antistress potential in *L. calbasu* fingerlings. Serum glucose (Fig. 1b) and serum cholesterol (Fig. 1c) followed a similar trend before and after acid stress. These protective responses and biochemical changes indicate the antistress activity of *Z. officinale* acetone extract which may be attributed to the effect of the extract on the central nervous system or endocrines, or the generation of free radicals (Kumar *et al*., 2005).

In contrast to the above parameters, serum triglycerides level significantly decreased ($p<0.05$) after acid stress in all the treatments (Fig. 1d). However supplementation of 0.5% as well as 5% levels of *Z. officinale* acetone extract in fish diet showed significant enhancement ($p<0.05$) in the level of triglycerides. Triglycerides, unlike cholesterol, recorded a decrease because triglycerides act as a rapid source of energy during stress conditions. There appears to be a close relationship between catecholamines and triglygerides (Mungantiwar *et al*., 1997). Therefore, it could be suggested that the decrease in the level of serum triglycerides is mediated through adrenal medullary secretions and through activation of sympathetic nervous system.

Thyroid hormone levels are affected by both acute and chronic stress in fish. In the present study, T_3

(Fig. 1e) and T_4 (Fig. 1f) concentrations in the serum were significantly ($p<0.05$) elevated in *L. calbasu* fed with control diet after exposure to pH 5.5, which could be due to the influence of cortisol on thyroid hormone. Gradual decrease in T_3 and T_4 concentrations were observed in all the treatment groups as *Z. officinale* acetone extract level increased, both pre- and post-acid stress, which may be attributed to the antistress property of *Z. officinale* extract. Present study showed parallel relationship between cortisol hormone and thyroid hormones as both hormone levels increased post-stress in control groups and which gradually decreased in all the treatment groups. Brown *et al*. (1991) reported that exposure to acid stress increased plasma cortisol and it has been documented that cortisol cause a decrease in T_4 to T_3 conversion (Eales and MacLatchy, 1989). Plasma T_3 levels are reduced in trout chronically exposed to low pH and aluminium (Brown *et al*., 1984).

membrane due to steroidal storms, which occur during stress (Nayanatara *et al*., 2009).

Oxidative stress parameters

SOD catalyses the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide. Subsequently, catalase (CAT) activity decomposes this hydrogen peroxide into oxygen and water, and this constitutes a crucial part of the cellular antioxidant defence mechanism. In general, stress causes oxidative damage to cells, which overcomes that effect through the increased activity of antioxidative enzymes (SOD and CAT). In the present study, increase in SOD and CAT activity in liver, muscle and gill of *L. calbasu* fingerlings in response to acid stress was observed (Table 3). An increase in SOD and CAT activity in liver, muscle, and gill after exposure

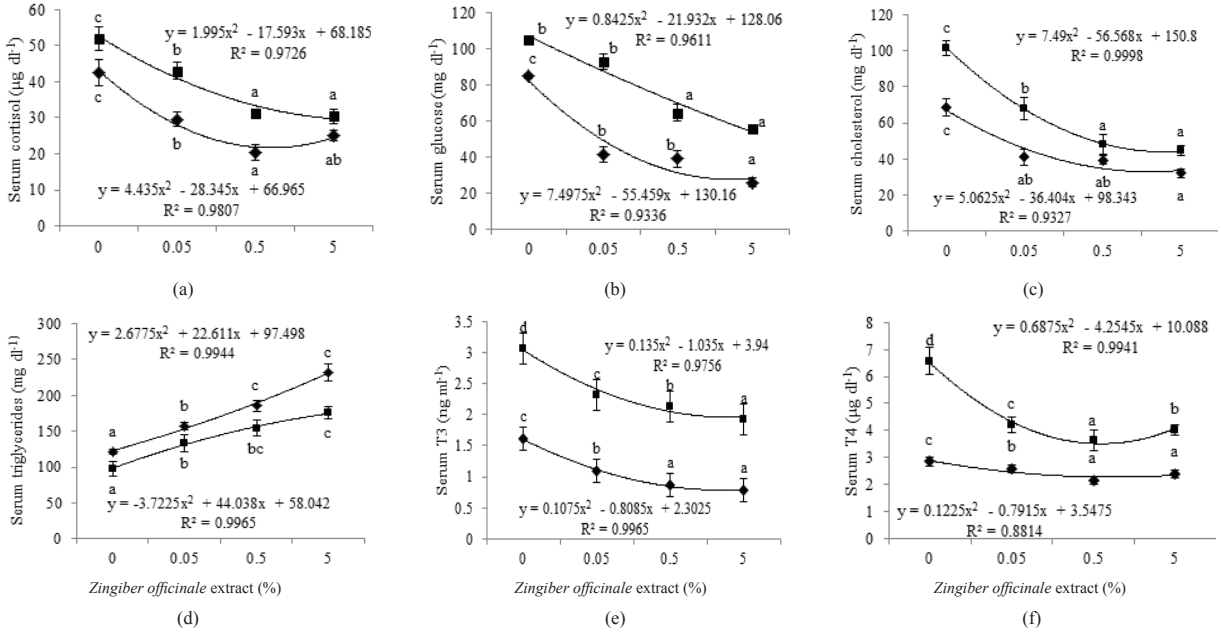

Fig. 1. Serum levels of (a) cortisol, (b) glucose, (c) cholesterol, (d) triglycerides, (e) T3 and (f) T4 in *L. calbasu* fed diets containing different levels of *Z. officinale* acetone extract before and after exposure to acid stress. = before and ■ = after acid stress. Values bearing different alphabets significantly differ ($p<0.05$).

ALT and AST are markers of hepatic functional status as they indicate parenchymal liver damage. The study of different enzyme activities such as ALT, AST and ALP has great value in the assessment of clinical and experimental liver damage (Vaishwanar and Kowale, 1976). In the present study, induction of acid stress led to raise the serum ALT, AST and ALP levels (Table 2). However, this pattern was reversed by *Z. officinale* acetone extract treatment in all the three treatments and produced a significant reduction ($p<0.05$) in AST and ALP levels, as compared to controls both before and after acid stress whereas, no significant effect was observed in serum ALT level before acid stress. ALT, AST and ALP levels increased in acid stress group possibly because of the alteration in the membrane permeability which might occur in the cells during stress. These changes also represent a functional alteration in the cell

to acid stress possibly indicates a role in O_2^- scavenging (Parihar *et al*., 1997; Shivendra *et al*., 2011). However, the activity was higher in high dietary *Z. officinale* extract–fed groups, indicating the defence response of *Z. officinale* to acid stress through the increased activity of SOD and CAT. In the present study, 0.5% and 5% levels showed higher SOD and CAT activity in stressed fish which is supported by the finding of Aruoma *et al*. (1997) and Valko *et al.* (2004), who reported that ginger extract can control the quantity of free radicals and the peroxidation of lipids *in vitro*. Thus, it could prevent or decrease he damage caused by free radicals, which, attack biological macromolecules such as lipids, proteins and DNA. By averting the oxidation of the phospholipids of cell membranes, the specific cell permeability is preserved and the cell metabolism is not

Table 2. Activity of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in serum of *L. calbasu* before and after acid stress fed with diets containing different levels of *Z. officinale* acetone extract at the end of experiment

Parameters	Acid stress	Treatments			
		Control (0%)	T_1 (0.05%)	T_2 (0.5%)	T_3 (5%)
ALT[1]	Before stress	32.12^{X}±6.19	24.99^{X}±1.44	21.62^{X}±2.06	21.95^{X}±0.50
	After stress	70.72^{Yc}±5.89	56.87^{Ybc}±1.47	34.18^{Ya}±6.48	48.33^{Yab}±1.77
AST[2]	Before stress	42.05^{Xb}±7.41	33.55^{Xb}±3.95	26.51^{Xab}±1.94	15.95^{Xa}±0.54
	After stress	72.19^{Yc}±2.06	55.10^{Yb}±4.42	45.67^{Yb}±1.47	21.51^{Ya}±0.88
ALP[3]	Before stress	303.14^{Xc}±16.78	266.96^{Xbc}±10.38	213.35^{Xab}±21.01	176.89^{Xa}±9.55
	After stress	589.00^{Yc}±16.46	313.84^{Ya}±5.49	363.70^{Yb}±10.94	284.30^{Ya}±14.02

Values represent mean ± SE
Mean values in a row under each parameter bearing different superscripts (a, b, c, d) differ significantly ($p<0.05$)
Mean values in a column (before and after heat shock) under each parameter bearing different superscripts (X and Y) differ significantly ($p<0.05$).
[1]ALT (Ul^{-1}) =ΔA/Min X 1768 (* = constant), [2]AST (Ul^{-1}) = ΔA/Min x1768* (* = constant) , [3]ALP (Ul^{-1}) =ΔA/min x 3300* (*= constant)*

Table 3. Activity of superoxide dismutase (SOD), catalase (CAT) and glutathione S transferase (GST) in different tissues of *L. calbasu* before and after acid stress at the end of experiment

Parameters	Tissues	Acid stress	Treatments			
			Control (0%)	T_1 (0.05%)	T_2 (0.5%)	T_3 (5%)
CAT[1]	Liver	Before stress	0.31^{Xa}±0.03	0.61^{Xb}±0.07	0.91^{Xc}±0.07	0.65^{Xb}±0.03
		After stress	0.62^{Ya}±0.03	0.80^{Yb}±0.07	1.60^{Yd}±0.03	1.28^{Yc}±0.01
	Muscle	Before stress	0.31^{Xa}±0.01	0.52^{Xab}±0.09	1.00^{Xb}±0.19	0.82^{Xb}±0.13
		After stress	0.63^{Ya}±0.02	0.88^{Yab}±0.11	1.11^{Yb}±0.13	1.73^{Yc}±0.04
	Gill	Before stress	0.27^{Xa}±0.01	0.57^{Xb}±0.04	0.60^{Xb}±0.12	0.65^{Xb}±0.02
		After stress	0.59^{Ya}±0.09	0.98^{Ya}±0.01	1.53^{Yb}±0.24	1.01^{Ya}±0.02
SOD[2]	Liver	Before stress	9.21^{Xa}±0.77	11.87^{Xab}±0.59	11.08^{Xab}±0.85	13.54^{Xb}±0.02
		After stress	10.67^{Ya}±0.56	13.13^{Yab}±0.54	14.52^{Yb}±1.39	16.02^{Yb}±0.56
	Muscle	Before stress	13.49^{Xa}±0.22	18.80^{Xab}±0.43	21.92^{Xb}±3.93	23.83^{Xb}±3.29
		After stress	14.52^{Ya}±0.52	22.65^{Yb}±0.96	24.16^{Yab}±0.61	25.49^{Yb}±0.54
	Gill	Before stress	24.06^{X}±0.85	30.43^{X}±0.39	27.04^{X}±4.19	28.78^{X}±0.78
		After stress	35.63^{Y}±1.09	31.95^{Y}±0.41	29.74^{Y}±3.06	35.60^{Y}±1.09
GST[3]	Liver	Before stress	0.41^{Xa}±0.02	0.63^{Xab}±0.03	0.69^{Xb}±0.16	0.76^{Xb}±0.05
		After stress	0.90^{Ya}±0.06	2.61^{Yb}±0.04	3.51^{Yc}±0.17	4.54^{Yd}±0.22
	Muscle	Before stress	0.55^{Xa}±0.01	0.98^{Xb}±0.01	2.24^{Xd}±0.11	1.30^{Xc}±0.04
		After stress	1.55^{Ya}±0.05	3.14^{Yab}±0.50	3.85^{Yb}±0.49	2.69^{Yab}±0.55
	Gill	Before stress	2.42^{Xa}±0.12	4.29^{Xb}±0.61	4.88^{Xb}±0.08	5.70^{Xb}±0.64
		After stress	3.68^{Ya}±0.15	5.83^{Yb}±0.34	6.34^{Yb}±0.05	8.01^{Yc}±0.12

Values represent mean ± SE
Mean values in a row under each parameter bearing different superscripts (a, b, c, d) differ significantly ($p<0.05$)
Mean values in a column (before and after pH stress) under each parameter bearing different superscripts (X and Y) differ significantly ($p<0.05$).
*[1]Activities expressed as U min^{-1} mg^{-1} protein = inhibition %/50x sample vol x mg protein (inhibition %= OD blank-change in OD/min/OD blank*100)*
[2]Activities expressed as µmol H_2O_2 decomposed min^{-1} mg^{-1} protein = OD diff/min x 1000/sample x 43.6 x mg protein
[3] micro moles of CDNB-GSH conjugate formed min^{-1} mg^{-1} protein=OD x reaction mix vol. x1000/9.6 x total time x mg protein x sample volume

disturbed. By protecting deoxy ribose from aggressive OH radicals, ginger extract could prevent DNA damage (Valko *et al*., 2004).

In the present study, dietary *Z. officinale* acetone extract levels significantly affected ($p<0.05$) the activity of GST in liver, muscle and gill of *L. calbasu* fingerlings both before and after acid stress (Table 3). The acid stress increased the GST activity in liver, muscle and gill. The GST activity also decreased significantly ($p<0.05$) in the tissues like liver, muscle and gill of *L. calbasu* both before and after acid stress. However, upon treatment with *Z. officinale* acetone extract, GST activity increased significantly ($p<0.05$) compared with control groups. The increased activity of GST observed in the fish exposed to acid stress in liver, muscle and gill may be because of the inactivation caused by reactive oxygen species (ROS) (Andallu and Vardacharyulu, 2003). The significant recoveries in the activities of these enzymes (CAT, SOD and GST) in stressed fish with supplementation of *Z. officinale* extract reflect the antioxidant potential of this herbal preparation.

The results of the present study showed that *Z. officinale* acetone extract supplementation in the diets of *L. calbasu* at levels of 0.5% and 5%., contributed to reduction in levels of serum cortisol, serum glucose, serum cholesterol, triglycerides, T3 and T4 and a marked improvement in production of oxidative stress enzymes in experimental fish exposed to acid stress. The extract seems to have no toxicity as no mortality was reported up to 10 times the effective dose. From economic point of view, 0.5% is the optimum level for supplementation in feed. However, the application of *Z. officinale* extract in field condition requires detailed studies on the methods of administration and effective doses for different age groups of fish. Further work needs to be carried out to isolate and characterise the active principles of *Z. officinale* acetone extract as well as to delineate its specific properties.

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