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Role of *Achyranthes aspera* (Linn.) leaf extract in the regulation of behavioural and metabolic responses in Walking Catfish, *Clarias batrachus* (Linnaeus, 1758)

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Abstract

Use of herbal supplements in aquaculture and fish industry is gaining attention to fulfil the growing nutritional demand of the increasing population worldwide. *Achyranthes aspera* L. commonly known as Chirchita, is an annual herb growing as a weed globally. It has occupied a pivotal position in Indian culture and folk medicines and is commonly used by traditional healers for the treatment of various diseases. In the present study, an investigation was made to find out the impact of aqueous leaf extract of *Achyranthes aspera* on behavioural and various metabolic responses in *Clarias batrachus*. Intramuscular administration of aqueous extract (0.5 ml; equivalent to dose of 300 mg/kg body weight) was given; and opercular movement was counted at 0h, 1h, 2h and 24h, which showed a significant increase of 96.49%, 84.63%, 62.64% and 40.66% respectively. After administration of aqueous extract daily for 10 days, serological analysis of Triiodothyronine (T_3), Thyroxine (T_4) and Thyroid-stimulating hormone (TSH) was done, which showed a significant increase at 5%, 0.1% and 1% level respectively. A significant decrease ($p<0.05$) in T_3/T_4 ratio was also observed. Body weight was also decreased significantly ($p<0.05$). Effect on nitrogen metabolism was evident showing significant decrease in serum uric acid ($p<0.001$) and increase in serum urea ($p<0.001$) level. Significant decrease in serum calcium ($p<0.001$) level was also observed. It may be concluded that the aqueous leaf extract of *Achyranthes aspera* had thyrostimulatory, hypocalcemic and hypouricemic properties which can influence the growth, metabolism, osmoregulation and reproduction in fishes.

Key words : *Achyranthes aspera, Clarias batrachus,* TSH, behavioural response, hypouricemic.

Fish is a potential and easily accessible source of high quality protein, vitamin D, selenium, omega-3 fatty acids and other nutrients. There is a constant increase in consumer demand for fish products, while wild fish stocks are rapidly declining due to overfishing. According to previous reports made by Food and Agriculture Organizations of the United Nations (FAO), 47 % of global fish stocks are fully exploited and another 18 % are reported as over exploited. Although several growth promoters and hormones have been tested for enhancing feed conversion efficiency and increasing the fish culture productivity, plant based safe and effective dietary supplements and additives; such as, saponins, flavonoids, essential oils, alkaloids, triterpenoids and phytoandrogens as growth promoting and endocrine modulating agents to enhance the life activity, health and immune system of farm fish have drawn attention of researchers[3]. In support of "back to nature" concept by discouraging the use of synthetic pharmaceutical products, including antibiotics, hormones and growth promoters, the role of plant extracts in disease management, growth promotion and improvement of immune conditions are extensively being studied by researchers across the globe[12,21]. Dietary supplementation with *Papaveraceae*[6] and *Origanum vulgare*[17] have been reported to exert positive impacts on growth, feeding, nutritional quality and biochemical indices of experimental fish. *Achyranthes aspera* Linn. (Prickly Chaff-flower, Amaranthaceae) is an erect and much branched diffuse herb found throughout India along roadsides and waste places. The plant has occupied a pivotal position in Indian culture and folk medicine and has been reported to have potential health benefits in terrestrial experimental animal models as well as human[14]. Supplementation with leaf, root and seed extracts of *Achyranthes aspera* have been reported to modulate immune activity in different experimental fishes[1,2,19]. Different parts of *Achyranthes aspera* have been reported to be rich in phytochemicals like alkaloids, flavonoids, saponins, steroids, tannins, cardiac glycosides and terpenoids[9] and have been reported to have anti-parasitic, larvicidal, wound healing, hypoglycemic, hypolipidemic, prothyroidic, analgesic and antipyretic activities[22,23]. In the present investigation, attempts have been made to investigate the acute effect of extract as well as its effect on metabolism of nitrogen, calcium and thyroid hormones in *Clarias batrachus*.

Preparation of leaf extract :

Fresh and healthy leaves of *Achyranthes aspera* were collected locally, washed, shade-dried and powdered. Aqueous leaf extract was prepared using Soxhlet apparatus from dried leaf powder[13]. The solvent was evaporated using hot water bath and extracted dried powder was used to prepare an aqueous solution for final administration.

Determination of dose :

On the basis of some previous reports, Sub-lethal concentration of the *Achyranthes aspera* was estimated[27]. And a dose of **300mg/kg body weight** leaf extract was taken as effective dose for the study for 10 days.

Experimental design and treatment :

Healthy, live *Clarias batrachus* were purchased from local market in Ranchi, Jharkhand and were acclimatized to the laboratory conditions for 14 days prior to experimentation. Fish were fed *ad libitum* with fish food purchased from market. Water in the aquarium was changed on alternate days, with regular siphoning off the excreta and left over feed. Before dividing the fish in separate groups, average weight of each fish was recorded. Fish were divided into two groups of (n=7) each. Group I fish were intramuscularly[7] administrated with equivalent amount of distilled water (0.5 ml) daily for 10 days and served as sham-control. In Group II (Extract- treated), fish were intramuscularly administrated with aqueous leaf extract of *Achyranthes aspera* at the dose of 300 mg/kg body weight (0.5 ml extract), daily for 10 days.

Behavioural Observations :

Time dependent acute stressful effect was studied by counting the number of opercular beats using stopwatch after administration of dose at 0, 1, 2 and 24 hr and the frequency of opercular beat was calculated.

Frequency of opercular beat = No. of beats / Minute.

Sample collection :

On the termination of experiment, fish were fasted overnight. They were weighed before sacrifice and blood was collected through cardiac puncture. The blood was allowed to clot and was centrifuged to get the serum. The serum samples were kept at -20°C for further analysis.

Biochemical estimations :

Serum samples from control and treated group were analyzed for level of thyroid hormones, uric acid, urea and calcium using fully auto biochemistry LWC 100 Germany.

Statistical Analysis :

Data were expressed as the arithmetic mean ± standard deviation (SD). Statistical analysis was done using student's t-test, and p-value of 5 % and less was considered as significant.

Table-1. Effects of *Achyranthes aspera* aqueous leaf extract (300 mg/kg body weight) on frequency of opercular beat per minute in *Clarias batrachus*.

	Control	After Administration of leaf extract			
		0 hr.	1 hr.	2 hr.	24 hr.
Frequency of Opercular beat (Per minute)	25.7 ± 2.983	50.5 ± 5.226**	47.45 ± 3.803**	41.8 ± 3.888**	36.15 ± 6.998
% increase from control		96.49%	84.63%	62.64%	40.66%

**p<0.01

Table-1 showed that there was an immediate response to the administration of *Achyranthes aspera* leaf extract. The frequency of opercular beat was increased significantly by 96.49%, 84.63% and 62.64% from the control condition at 0, 1 and 2 hr respectively ($p<0.01$). There was evident increase in frequency of opercular beat even after 24 hr of administration (40.66%), but the increase was insignificant.

Table-2. Effects of *Achyranthes aspera* aqueous leaf extract (300 mg/kg body weight) daily for 10 days on Body Weight and Serum level of T_3, T_4, TSH, Uric Acid, Urea and Calcium in *Clarias batrachus*.

Parameters	Control (Distilled Water)	(Extract-treated)	% Change (+) Increase/(-) Decrease
Body Weight (g)	70 ± 3.319	60 ± 2.200*	- 14.28
T_3 (ng/dl)	23.2 ± 1.80	31.0 ± 1.95*	+ 33.62
T_4 (Ug/dl)	1.20 ± 0.03	1.90 ± 0.05***	+ 58.33
T_3/T_4 Ratio	19.33 ± 0.99	16.31 ± 0.61*	-15.62
TSH (ulU/ml)	0.12 ± 0.011	0.20 ± 0.023**	+ 66.66
Uric Acid (mg/dl)	1.2 ± 0.08	0.5 ± 0.02***	- 58.33
Urea (mg/dl)	6.0 ± 0.08	9.1 ± 0.12***	+ 51.66
Calcium (mg/dl)	10.6 ± 0.12	2.4 ± 0.05***	- 77.35

*$p<0.05$, **$p<0.01$, ***$p<0.001$

Table-2 showed that on daily administration of aqueous leaf extract of *Achyranthes aspera* for 10 days, there was a significant decrease in body weight ($p<0.05$), Serum Uric acid ($p<0.001$) and Calcium ($p<0.001$) levels as well as T_3/T_4 ratio ($p<0.05$). On the contrary, there was significant increase in serum T_3 ($p<0.05$), T_4 ($p<0.001$), TSH ($p<0.01$) and Urea ($p<0.001$) levels.

In the present investigation, a significant increase in the opercular movement was observed immediately after the intramuscular administration of leaf extract. It may be due to sudden response to shock[11]. Fish in general have low metabolic rate in resting phase. Increase in opercular movement indicated the respiratory stress to meet up the increased oxygen demand or the increased metabolic rate[26]. Behavioural abnormalities in a subject is dependent on several extrinsic and intrinsic factors[20]. An insignificant difference between opercular movement of control fish and treated fish after 24 hr of extract treatment showed a certain degree to adaptation to the exposure. Corroborating with the present finding, Pandit and Ghosh, 2005[15] reported a similar pattern of opercular movement in *Channa punctatus* after anaesthetization with benzocaine.

In teleosts, the thyroid gland consists of a large number of follicles diffused around the ventral aorta and afferent branchial arteries. Unlike most of the air- breathing fishes, the thyroid gland in *Clarias batrachus* is present in form of condensed patches but is without a capsule. Heterotropic presence of thyroid follicles in head kidney, brain, eye, oesophagous and spleen regions have also been reported in some teleosts[10]. Thyroid hormones (triiodothyronine, T_3 and thyroxine, T_4) have been reported to be involved in a large number of biological processes including metabolism, growth, development and reproduction in fishes[18]. T_4 is synthesized primarily in thyroid gland and is directly secreted into the blood. T_3 is secreted by thyroid gland in a very small amount (~13%) and the majority is formed in the peripheral tissues, mainly in liver, where T_4 is converted into T3 by the activity of type-I 5'-iodothyronine monodeiodinase (5'-DI)[24]. The scientific exploration of effects of administration with *Achyranthes aspera* extract on thyroid profile can provide an insight for its effect on several other metabolic responses also.

Table-2 showed a significant increase in serum T_3 as well as T_4 level. Treatment with the extract resulted in a substantial increase (58.33%) in the level of T_4 in serum; but the increase in the level of T_3 was (33.62%) only. This increase in T_4 concentration in the extract-treated animal suggested that the plant extract primarily stimulates thyroid gland to synthesize and secrete T_3 and T_4. A significant decrease in T_3/T_4 ratio was observed in the treated group suggesting that the extract has little or no role in the extrathyroidal conversion of T_4 to T_3. The significant increase in serum T_3 concentration may be due to stimulation of intrathyroidal hormone synthesis rather than extrathyroidal conversion of T_4 to T_3. Similar effects have been found in plant extracts of Ashwagandha in mice[13]. Many herbal extracts have been reported to have regulatory effects on thyroid function in experimental animal models[5]. Aqueous leaf extract of *Achyranthes aspera* have been reported to increase the serum level of T_3, T_4 as well as T_3/T_4 ratio in male rats[25]. A significant increase in TSH level may also be due to its effect on pituitary gland to stimulate the synthesis of thyroid hormones. Increase in T_3 and T_4 level, increases the Basal Metabolic Rate (BMR), resulting a decrease in body weight ($p<0.05$).

After administration of the extract for 10 days, a significant decrease in the serum uric acid level as well as a significant increase in the serum urea level was observed, suggesting the probable effect of the extract on the nitrogen metabolism in the fish. This increase in serum urea level, either by uricolysis (breakdown of uric acid) or by OUC (Ornithine-Urea Cycle), could be a defence against ammonia toxicity in air-breathing fishes[4,8]. A significant decrease in serum calcium level may be either due to uptake of calcium from the blood or due to excess excretion of calcium. The thyroxine have been reported to have suppressive effects on estrogen induced hypercalcemia in different fish species[16]. Thyrostimulatory activity of the extract treatment can be beneficial in regulation of thyroid function alterations induced by environmental changes or exposure to toxicants.

It can be concluded that the aqueous leaf extract of *Achyranthes aspera* has no toxic effects on the survival of the fishes and also had thyrostimulatory, hypouricemic and hypocalcemic properties which may positively influence the growth, survival, reproductive and other metabolic responses. Dietary and other mode of supplementation with *Achyranthes aspera* extracts and its study of its mechanism of action needs further investigation.

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