

Serum biochemical and histological studies in common carp (*Cyprinus carpio*) fed with pointed gourd (*Trichosanthes dioica*)

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ABSTRACT

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The effect of graded levels of pointed gourd (*Trichosanthes dioica*) feeding on biochemical and histological alterations in *Cyprinus carpio* was investigated. Four diets at 0, 5, 10 and 15% inclusion levels of pointed gourd fruit (PGF) paste designated as groups G0, G1, G2 and G3 respectively. Fish fed with 15% PGF paste manifested greater reduction in the levels of total serum glucose, aspartate amino transferase, alanine amino transferase, creatinine and significant ($p < 0.05$) increase in total serum protein, albumin and globulin level. The changes were significant between the groups and within the group at different days post treatment (DPT). In all the groups at all the intervals, i.e. 14th, 35th and 63rd DPT, liver revealed severe vacuolar degeneration of hepatocytes, swelling of hepatocytes leading to loss of sinusoidal spaces. At many places, hepatocytes were showing coagulative necrosis. In spleen and head kidney, no histological alterations could be seen in any groups. The results indicated that *Cyprinus carpio* can be fed pointed gourd fruit in basal feed up to 15% inclusion level without any negative effect on the health status of the fish.

Keywords: Biochemical, common carp, histology, *Trichosanthes dioica*

INTRODUCTION

Aquaculture is one of the fastest growing food production systems in the world, which has emerged as an industry possible to supply protein rich food throughout the world¹. Currently varieties of marine and brackish water species of fin fishes and shellfishes are cultured in over one million hectares for food in several Asian countries². Fish feeds account for about 70% of aquaculture operations, and most fish farmers do not make use of standard fish feed due to high cost of production. Feed cost constitutes one of the major problems of aquaculture³. The increasing price of feed is considered one of the most important factors that limit profitability in aquaculture systems, caused mainly by the cost of fish meal used as a primary source of protein⁴. As a result, there is a constant search for alternative protein sources for aquaculture diets.

Non-conventional Feed Resources (NCFRs) are feeds that are not usually common in the market and are not the traditional ingredients for commercial fish feed production⁵ and these include host of plants and animals by-products. Plant proteins are considered among the most viable alternatives to replace fish meal, since their production is only limited by the availability of land, assuming that the environmental conditions for the crops are adequate.

Trichosanthes dioica Roxb. (TD) commonly known as parwal, belongs to family *Cucurbitaceae*. Fruits of TD are

used as vegetable and considered as important delicacy. Its fruits are a rich source of vitamin C and minerals such as 9.0 mg Mg, 2.6 mg Na, 83.0 mg K, 1.1 mg Cu, and 17.0 mg S per 100 g. In addition, fruits and other parts such as leaves and tender shoots have been used in the indigenous system of medicine since ancient times⁶. According to ayurveda, the TD is also used for bronchitis, biliousness, cancer, jaundice, liver affections (enlargement), cough, and blood diseases. It is also used as antipyretic diuretic, cardiogenic and laxative⁷. Many a times, farmer is left with surplus TD and also TD which is unfit for human consumption. So an attempt has been made to include the TD in the basal ration of fish to replace the conventional feed ingredients. Keeping in view, the experiment was planned to study the adverse effect of regular feeding of whole fruits of TD specifically on some serum biochemical parameters and histology of liver, spleen and head kidney.

MATERIALS AND METHODS

Experimental fish

The experiment was conducted at College of Fisheries, GB Pant University of Agriculture and Technology, Pantnagar (Uttarakhand). The total duration of experiment was of 63 days. The experiment was conducted from April, 2015 to June, 2015. Experimental fishes were obtained from Instructional Fish Farm, College of Fisheries, Pantnagar. Eighty fishes of average weight 250g of both sexes were randomly divided into 4 groups of 20 fish each. Each group was set in duplication

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with 10 fishes per tank and were acclimatized for one week before the start of feeding.

Experimental plant material

Fresh pointed gourd fruit was purchased from the local vegetable market of Pantnagar. It was identified by the Department of Vegetable Sciences, College of Agriculture, GBPUAT, Pantnagar. The collected fruit was washed properly with tap water to remove dirt. The fruit was grinded using a laboratory grinder and was kept in the fridge at 4°C till use.

Formulation of experimental diets

The basal fish feed includes Rice bran and Mustard Oil cake. Fruit paste was included at the rate of 0%, 5%, 10% and 15% of the daily fish diet which was fed to groups G0, G1, G2 and G3, respectively. The fish were fed at the rate of 5% of their body weight, daily, in two equal portions, in the morning (9.00hr) and evening (16.00hr).

Biochemical estimation

At 0th, 14th, 35th and 63rd days post treatment (DPT), blood samples from 5 fish from each experimental group was drawn and serum was separated. The separated serum was stored in refrigerator at 4°C till further use. Separated serum was used for estimation of total serum protein, albumin, globulin, creatinine and serum enzymes such as aspartate amino transferase and alanine amino transferase (Transasia Bio-medicals Ltd., Solan, HP, India) and total serum glucose (Span Diagnostics Ltd., Surat, India).

Pathological study

At 14th, 35th and 63rd DPT, 3 fish from each group were sacrificed using humane procedures. All the sacrificed fishes were subjected to detailed post mortem examination and the gross lesions were duly recorded. Representative samples of the liver, spleen and head kidneys of each fish were collected in 10% normal buffered formalin for fixation. The collected tissues were processed for histopathological examination using standard procedures⁸. Briefly the procedure is as follows. The collected tissues were washed overnight to remove the formalin. Tissues were dehydrated using ascending grades of alcohol, cleared in xylene, impregnated with paraffin wax and paraffin blocks were made. Sections were cut at 4μ thickness and stained with haematoxylin and eosin (H&E) for histologic examination under the light microscope.

Statistical analysis

Data obtained during the course of investigation was statistically analysed by two-way analysis of variance (ANOVA) using Statistical Package for Social Sciences 2006 version 16.0 (SPSS). Means of the samples were compared by Duncan multiple range test and the level of significance were tested at $P < 0.059$.

RESULTS

Total serum protein

Table 1 shows the total serum protein in different groups of experimental fishes at varying time intervals. It is evident from the table that, the mean total serum protein levels in group G0, varied between 2.560 ± 0.05 to 2.542 ± 0.10 and did not show significant variation. In groups, G1, G2 and G3 values of total serum protein ranged between 2.554 ± 0.09 to 4.877 ± 0.16 , from 2.561 ± 0.10 to 5.329 ± 0.09 and from 2.556 ± 0.06 to 6.728 ± 0.07 respectively. The values of total serum protein showed significant ($p < 0.05$) increase in all the groups as compared to G0 from 0th DPT to 63rd DPT.

When these values in different groups were compared at different time intervals, no significant variation was recorded at 0 DPT between any groups. There was a significant ($p < 0.05$) increase in the total serum protein at all time intervals (14th, 35th and 63rd DPT) where group G3 had highest values in comparison to group G0 followed by groups G2 and G1.

Serum albumin

The serum albumin values in different groups at various time intervals are presented in Table 2. In group G0, the mean serum albumin ranged from 1.215 ± 0.11 to 1.221 ± 0.11 with no significant variation from 0 DPT to 63rd DPT. In groups, G1, G2 and G3, the values ranged between 1.208 ± 0.09 to 2.428 ± 0.12 , 1.213 ± 0.12 to 2.910 ± 0.02 and 1.223 ± 0.05 to 3.428 ± 0.06 respectively. In all the groups, serum albumin increased significantly ($p < 0.05$) from 0th DPT to 63rd DPT. When these values were compared between different groups, no significant variation was recorded at 0 DPT between any groups. There was a significant ($p < 0.05$) increase in the serum albumin level in all the groups at all time intervals (14th, 35th and 63rd DPT) where group G1 showed least increase in albumin level followed by groups G2 and G3.

Serum globulin

Serum globulin level in different groups at varying time intervals is presented in Table 3. In group G0, the mean serum globulin ranged from 1.345 ± 0.05 to 1.368 ± 0.05 showing no significant difference from 0 DPT to 63rd DPT. Groups G1, G2 and G3 showed significant ($p < 0.05$) increase in serum globulin content from 0th DPT to 63rd DPT with the values ranging from 1.324 ± 0.08 to 1.826 ± 0.07 , 1.335 ± 0.08 to 2.364 ± 0.07 and 1.324 ± 0.10 to 3.517 ± 0.07 respectively.

When these values were compared at varying time intervals, between different treatments no significant variation was recorded at 0 DPT. There was a significant ($p < 0.05$) increase in the serum albumin level at all time intervals (14th, 35th and 63rd DPT). Least increase was recorded in group G1 followed by groups G2 and G3.

Table 1. Average (Mean±SE) total serum protein (g/dl) levels in different groups of experimental fishes at different time intervals.

DPT	Groups			
	G0	G1	G2	G3
0 th DPT	2.560±0.05 ^{Aab}	2.554±0.09 ^{Db}	2.561±0.10 ^{Da}	2.556±0.06 ^{Dab}
14 th DPT	2.584±0.11 ^{Ad}	2.808±0.13 ^{Cc}	3.158±0.13 ^{Cb}	3.699±0.05 ^{Ca}
35 th DPT	2.539±0.10 ^{Ad}	3.576±0.11 ^{Bc}	3.949±0.07 ^{Bb}	4.948±0.06 ^{Ba}
63 rd DPT	2.542±0.10 ^{Ad}	4.877±0.16 ^{Ac}	5.329±0.09 ^{Ab}	6.728±0.07 ^{Aa}

Different small letters (a, b, c and d) indicate significant ($P<0.05$) difference between groups at a particular day, whereas different capital letters (A, B, C and D) indicate significant ($P<0.05$) difference between days within a particular group.

Table 2. Average (Mean±SE) serum albumin (g/dl) levels in different groups of experimental fishes at different time intervals.

DPT	Groups			
	G0	G1	G2	G3
0 th DPT	1.215±0.11 ^{Ab}	1.208±0.09 ^{Dc}	1.213±0.12 ^{Dbc}	1.223±0.05 ^{Da}
14 th DPT	1.221±0.03 ^{Ad}	1.799±0.15 ^{Cc}	1.968±0.06 ^{Cb}	2.114±0.02 ^{Ca}
35 th DPT	1.222±0.06 ^{Ad}	2.138±0.13 ^{Bc}	2.409±0.07 ^{Bb}	2.988±0.02 ^{Ba}
63 rd DPT	1.221±0.11 ^{Ad}	2.428±0.12 ^{Ac}	2.910±0.02 ^{Ab}	3.428±0.06 ^{Aa}

Different small letters (a, b, c and d) indicate significant ($P<0.05$) difference between groups at a particular day, whereas different capital letters (A, B, C and D) indicate significant ($P<0.05$) difference between days within a particular group.

Table 3. Average (Mean±SE) serum globulin (g/dl) levels in different groups of experimental fishes at different time intervals.

DPT	Groups			
	G0	G1	G2	G3
0 th DPT	1.345±0.05 ^{Aa}	1.324±0.08 ^{Dc}	1.335±0.08 ^{Db}	1.324±0.10 ^{Dc}
14 th DPT	1.358±0.08 ^{Ad}	1.428±0.09 ^{Cc}	1.528±0.09 ^{Cb}	1.368±0.07 ^{Ca}
35 th DPT	1.3567±0.05 ^{Ad}	1.548±0.08 ^{Bc}	1.739±0.05 ^{Bb}	2.447±0.07 ^{Ba}
63 rd DPT	1.368±0.05 ^{Ad}	1.826±0.07 ^{Ac}	2.364±0.07 ^{Ab}	3.517±0.07 ^{Aa}

Different small letters (a, b, c and d) indicate significant ($P<0.05$) difference between groups at a particular day, whereas different capital letters (A, B, C and D) indicate significant ($P<0.05$) difference between days within a particular group.

Table 4. Average (Mean±SE) total serum glucose (mg/dl) levels in different groups of experimental fishes at different time intervals.

DPT	Groups			
	G0	G1	G2	G3
0 th DPT	80.249±0.07 ^{Ac}	80.254±0.10 ^{Abc}	80.251±0.11 ^{Aa}	80.259±0.08 ^{Aab}
14 th DPT	80.237±0.03 ^{Ad}	78.160±0.16 ^{Bc}	77.546±0.10 ^{Bb}	75.351±0.09 ^{Ba}
35 th DPT	80.253±0.05 ^{Ad}	74.552±0.12 ^{Cc}	73.396±0.07 ^{Cb}	70.462±0.05 ^{Ca}
63 rd DPT	80.304±0.04 ^{Ad}	70.578±0.11 ^{Dc}	68.931±0.39 ^{Db}	64.988±0.010 ^{Da}

Different small letters (a, b, c and d) indicate significant ($P<0.05$) difference between groups at a particular day, whereas different capital letters (A, B, C and D) indicate significant ($P<0.05$) difference between days within a particular group.

Total serum glucose

Total serum glucose level in different groups at varying time intervals is presented in Table 4. In group G0, the mean serum glucose content ranged from 80.249±0.07 to 80.304±0.04 and there was no significant variation from 0 DPT to 63rd DPT. In all the groups, G1, G2 and G3 there was a significant ($p<0.05$) decrease in

the serum glucose content from 0th DPT to 63rd DPT with the values varying from 80.254±0.10 to 70.578±0.11, 80.251±0.11 to 68.931±0.39 and 80.259±0.08 to 64.988±0.010, respectively.

When these values were compared between different groups, no significant variation was recorded at 0th DPT. There was a significant ($p<0.05$) decrease in the serum

Table 5. Average (Mean±SE) serum creatinine (mg/dl) levels in different groups of experimental fishes at different time intervals.

DPT	Groups			
	G0	G1	G2	G3
0 th DPT	0.573±0.002 ^{Aa}	0.576±0.003 ^{Aa}	0.575±0.001 ^{Aa}	0.572±0.002 ^{Aa}
14 th DPT	0.570±0.003 ^{Aa}	0.558±0.002 ^{Bb}	0.530±0.001 ^{Bc}	0.502±0.002 ^{Bd}
35 th DPT	0.572±0.002 ^{Aa}	0.478±0.002 ^{Cb}	0.451±0.001 ^{Cc}	0.423±0.001 ^{Cd}
63 rd DPT	0.573±0.002 ^{Aa}	0.420±0.002 ^{Db}	0.392±0.001 ^{Dc}	0.341±0.001 ^{Dd}

Different small letters (a, b, c and d) indicate significant ($P<0.05$) difference between groups at a particular day, whereas different capital letters (A, B, C and D) indicate significant ($P<0.05$) difference between days within a particular group.

Table 6. Average (Mean±SE) serum AST (IU/L) activity in different groups of experimental fishes at different time intervals.

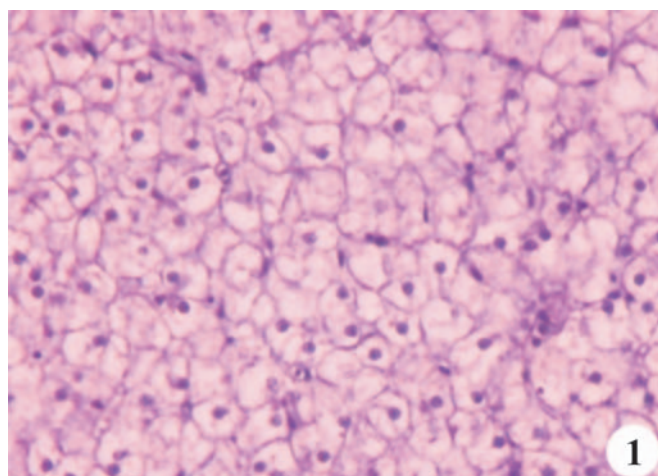
DPT	Groups			
	G0	G1	G2	G3
0 th DPT	55.263±0.15 ^{Aa}	55.264±0.17 ^{Aa}	55.268±0.11 ^{Aa}	55.261±0.12 ^{Aa}
14 th DPT	55.246±0.13 ^{Ad}	53.030±0.08 ^{Bc}	50.812±0.22 ^{Bb}	49.158±0.29 ^{Ba}
35 th DPT	55.254±0.06 ^{Ad}	51.090±0.25 ^{Cc}	47.480±0.17 ^{Cb}	44.410±0.17 ^{Ca}
63 rd DPT	55.261±0.04 ^{Ad}	47.216±0.14 ^{Dc}	43.450±0.18 ^{Db}	39.188±0.15 ^{Da}

Different small letters (a, b, c and d) indicate significant ($P<0.05$) difference between groups at a particular day, whereas different capital letters (A, B, C and D) indicate significant ($P<0.05$) difference between days within a particular group.

Table 7. Average (Mean±SE) serum ALT (IU/L) activity in different groups of experimental fishes at different time intervals.

DPT	Groups			
	G0	G1	G2	G3
0 th DPT	21.256±0.16 ^{Ab}	21.264±0.15 ^{Aa}	21.251±0.10 ^{Ab}	21.260±0.12 ^{Aa}
14 th DPT	21.234±0.03 ^{Ad}	20.574±0.11 ^{Bc}	19.412±0.16 ^{Bb}	18.276±0.26 ^{Ba}
35 th DPT	21.212±0.03 ^{Ad}	18.440±0.17 ^{Cc}	16.462±0.19 ^{Cb}	15.228±0.19 ^{Ca}
63 rd DPT	21.238±0.04 ^{Ad}	15.132±0.15 ^{Dc}	12.902±0.16 ^{Db}	10.884±0.10 ^{Da}

Different small letters (a, b, c and d) indicate significant ($P<0.05$) difference between groups at a particular day, whereas different capital letters (A, B, C and D) indicate significant ($P<0.05$) difference between days within a particular group.

**Fig.1.** Microphotograph of liver showing vacuolar degeneration, loss of sinusoidal spaces and few cells showing coagulative necrosis. H&E ×200.

glucose level at all time intervals (14th, 35th and 63rd DPT) where groups G1 and G3 showed minimum and maximum decrease in the values, respectively. The decrease in total serum glucose content was significant between groups at 14th, 35th and 63rd DPT.

Serum creatinine

Serum creatinine content in different groups at varying time intervals is presented in Table 5. In group G0, the mean concentration of creatinine ranged from 0.573±0.002 to 0.573±0.002 and there was no significant variation from 0th DPT to 63rd DPT. Groups G1, G2 and G3 showed significant ($p<0.05$) decrease in the serum creatinine content with values ranging from 0.576±0.003 to 0.420±0.002, 0.575±0.001 to 0.392±0.001 and 0.572±0.002 to 0.341±0.001 respectively.

When the mean creatinine values in different groups were compared at varying time intervals no significant

difference could be noted at 0 DPT between any groups. From 0th DPT to 63rd DPT, all groups showed significant ($p < 0.05$) decrease in serum creatinine with least decrease in groups G1 followed by groups G2 and G3.

Serum Aspartate aminotransferase (AST)/ Serum glutamic oxaloacetic transaminase

Serum AST activity in different groups of experimental fishes at varying time intervals is depicted in Table 6. In group G0, the mean AST activity ranged between 55.263 ± 0.15 to 55.261 ± 0.04 and there was no significant variation from 0th DPT to 63rd DPT. In all the groups, G1, G2 and G3, there was a significant ($p < 0.05$) decrease in the AST activity from 0th DPT to 63rd DPT, with the values varying between 55.264 ± 0.17 to 47.216 ± 0.14 , 55.268 ± 0.11 to 43.450 ± 0.18 and 55.261 ± 0.12 to 39.188 ± 0.15 , respectively.

When the mean AST values in different groups were compared at different time intervals, no significant variation was recorded at 0th DPT between any groups. All the groups showed significant ($p < 0.05$) decrease in the values from 14th DPT to 63rd DPT where maximum decrease in the values of serum AST was recorded in group G3 followed by groups G2 and G1.

Serum Alanine aminotransferase (ALT)/ Serum glutamic pyruvic transaminase

Serum ALT activity in different groups of experimental fishes at varying time intervals is depicted in Table 7. In group G0, the mean ALT activity ranged between 21.256 ± 0.16 to 21.238 ± 0.04 and there was no significant variation from 0 DPT to 63rd DPT. In all the groups, G1, G2 and G3 there was a significant ($p < 0.05$) decrease in the ALT activity with the values ranging between 21.264 ± 0.15 to 15.132 ± 0.15 , 21.251 ± 0.10 to 12.902 ± 0.16 and 21.260 ± 0.12 to 10.884 ± 0.10 respectively.

When the mean ALT values in different groups were compared at different time intervals, no significant variation was recorded at 0 DPT between any groups. All the groups showed significant ($p < 0.05$) decrease in the values of ALT from 14th DPT to 63rd DPT with Group G1 showed least decrease in values followed by groups G2 and G3.

Pathological studies

Histopathologically, liver revealed severe vacuolar degeneration of hepatocytes throughout the liver parenchyma, swelling of hepatocytes leading to loss of sinusoidal spaces in all the groups at all the intervals (14th, 35th and 63rd DPT). At many places, hepatocytes showed coagulative necrosis (Fig. 1). No changes were seen in spleen and kidneys in any group.

DISCUSSION

PGF diet resulted in significant increase in total

serum protein, albumin and globulin levels. Similar finding were also reported by others¹⁰ where they observed increase in total serum protein, albumin and globulin level by using neem in Nile tilapia (*Oreochromis niloticus*) and African cat fish (*Clarias gariepinus*). Rai *et al.*¹¹ fed the aqueous extract of *Trichosanthes dioica* fruits to rats at a dose of 1000mg/kg body weight daily once for 28 days resulted in increase in total protein. The values of serum glucose, creatinine, AST and ALT showed decreased with increase in concentration of the pointed gourd fruit paste supplementation. Results of this study showed that serum AST and ALT activities decreased significantly in the fish group fed on all levels of TD. These observations are in agreement with those of others^{12,13} who found that the enzyme activities (AST, ALT, and ALP) in serum of rats decreased significantly when were fed pointed gourd fruit extract. Decreased serum activity of AST and ALT might be due to decreased hepatocellular production or release of the enzymes¹⁴. TD helps the liver to maintain its normal function by accelerating the regenerative capacity of its cells. Hence, it has hepatoprotective activity. In the present study, total plasma glucose concentration reduced significantly in fish fed diets containing the different levels of PGF paste. These results are in accordance with those of other researches^{12,15} in which reduction of blood glucose in normal and STZ diabetic rats fed aqueous fruit extract of *T. dioica* was observed. Chandrasekhar *et al.*¹⁶ have also reported that pointed gourd possesses the medicinal property of lowering blood sugar level in rats. The values of serum glucose decreased significantly ($P \leq 0.05$) which may be due to hypoglycemic activity of pointed gourd and probably due to phyto-chemical constituents demonstrated in the fruit i.e. flavonoids, saponins, polyphenols, alkaloids and tannins¹³. Although the results of the present study showed a decrease in serum AST, ALT, creatinine and glucose levels but these values ranged within the normal limits of parameters in carps¹⁷.

It was concluded from the present study that the TD fruit can be incorporated in fish diet up to 15% of inclusion level without any deleterious effects on these parameters.

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