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Antibacterial and antidiabetic evaluation of bile content of *Catla catla* & *Labeo rohita*

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ABSTRACT

Fish is one of the popular health foods worldwide and has huge medicinal importance. Several traditional medical practitioners of Tripura claimed that bile content of fresh water fish may useful in diabetes. Therefore the present work was undertaken to evaluate antibacterial and antidiabetic activity the bile content of two major fresh water carps, Catla catla and Labeo rohita. Physicochemical characterization including stability study was also carried out for bile content. Moderate antibacterial activity was observed against two gram positive such as Bordetella pertusis, Staphylococcus aureus, and two gram negative like Eschericia coli and Vibrio cholerae bacterial strains. Oral and inraperitonial administration of fresh bile content of both fresh water carp possesses remarkable antidiabetic activity. Though mortality of animal is a major concern; mortality was less in case animal received diluted bile content. Though, further research is necessary for dose fixation and identification of active constituents.

Keywords: *Catla catla*, *Labeo rohita*, bile content, antibecterial, antidiabetic.

INTRODUCTION

The ancient wisdom of the people of every nation has been crystallized into different systems of traditional or folk medicine. Among the different traditional systems some are well established and known as codified systems of 'traditional medicine, supported by theories and rich experience and recorded in writing like Ayurveda, while non-codified traditional medicinal systems such as folk, tribal or indigenous medicine has merely been practiced and passed verbally from generation to generation, without any written record [1, 2]. Today ethnicity is as vibrant as any time in history and it is our responsibility to take the essential steps to getter the knowledge about the rich non-codified traditional medicine of the ethnic communities and use them as a lead in advancing the frontiers of science and technology.

Tripura is a small, beautiful hilly state of India known for its reservoirs of traditional knowledge. Tripura is rich in diversity of species, habitats and ecosystems. The state also rich in ethnic diversity and home of 19 ethnic communities including Tripuri, Riang, Jamatia, Chakma, Lepcha, Mogh, Garo, Kuki, Chaimol, Uchai, Halam,

Khasia, Bhutia, Lushai, Noatia, Bhil, Santal, Orang, Munda. According to the 2011 census the population of Tripura stands at 3671032, among them Bengalis represents almost 70% of population and the tribal populations represent almost 30% of Tripura's population [3, 4, 5]. Tribal people of Tripura, India are very much scientific in maintaining of their nutritional demand and thus in few findings it is noticed that they are devoid of many diseases which are even life threatening in few cases for non-tribal people living at Tripura. To explore the nutritional status of Tribal people of Tripura, and investigation on different dishes of ethnic communities of Tripura is the demand of this scientific era.

Fish is one of the most popular food items of the non-vegetarians. It is unique animal meat that is rich in all essential amino acids and fatty acids and many other nutrients. Approximately 75% of world populations are dependant directly or indirectly depends on fishes for protein food [6]. Fish contains protein (15-26%), fat (0.8-19.7%), minerals (0.9-2.2%), vitamin A, D, C and water (70-80%) [7]. Several researchers have proved the beneficial effect of fish in heart diseases, inflammatory diseases, mineral deficiency etc. An antioxidant ω-3-fatty acid is abundant in fish, which is also essential for healthy development of the eyes and brain. Recent studies also reported its importance in treatment or management of diabetes, cardiovascular diseases [6, 8, 9]. Several species of fresh water carps which are very copiously found in Tripura includes *Catla catla* (Katla), *Labeo rohita* (Rohu), *Labeo calbasu* (Kalibous), *Cirrhinus mrigala* (Mrigal) etc. The importance of fish in diet lies in the chemical composition of the flesh, which is rich in proteins and minerals like calcium, phosphorous, iron etc. We also observed that few traditional medical practitioners of tribal communities (locally known as auchai or kabiraj) of Tripura claimed the therapeutic action of other parts excluding flesh of the fish, such as bile of the fresh water fish in diabetes. The bile is known as *Bakhwl* in Tripuri community and they some time take this by frying with chilies and onion. Keeping this in view, the work was undertaken to carryout physicochemical characterization including stability study, antibacterial and antidiabetic evaluation of the bile content of two major fresh water carps, *Catla catla* and *Labeo rohita* that are very abundantly found in the market of Tripura, India.

MATERIALS AND METHODS

Collection of samples

The sample fishes used in this work were collected from the local water bodies. The carp, *Catla catla* (1–2 kg) and *Labeo rohita* (200–400 gm) were chosen as per availability in the market for the study. The bile was collected from intact gall bladder of two different fish species separately. The collected bile was then preserved at 0ºC for further study.

Experimental animals

Adult, healthy albino mice weighing between 25–30 g were used in antidiabetic experiments. The animals were maintained under standard laboratory conditions of temperature (20±2°C), relative humidity (50±15%), 12 h light–dark cycle, standard diet and water *ad libitum*. All the experiments on animals were carried out as per the guidelines of the institutional animal ethics committee.

Evaluation of physicochemical parameters

Physicochemical parameters of bile such as color, pH, specific gravity, R_f value were determined along with stability study. The colour observed by naked eyes, pH was measured by using digital pH meter (Eutech Instruments pH tutor Singapore) and specific gravity was determined by adopting a standard method [10]. R_f value of crude bile content was determined by TLC using silica gel G as stationary phase, various solvent and combinations as mobile phase, iodine vapors used for spot detection [11]. Further qualitative chemical tests for the presence of various organic and inorganic substances were also carried out by adopting the standard procedures [10, 12, 13].

Stability testing

Equal amount of freshly collected bile was taken in five different test tubes and kept at different elevated temperature (0º, 4º, 37.7º, 50º and at room temperature). Before subjecting to different temperature, the λ_{max} for each sample was determined with the help of colorimeter (Systronics photoelectric colorimeter 113) using sterile water as blank [11, 14]. Then samples taken out from different temperature and kept aside for a while so that the temperature of the samples comes to the room temperature and absorbance for each sample was recorded at different time intervals (0 h, 1 h, 2 h, 4 h, 24 h).

Antibacterial activity

In vitro antibacterial activity for the crude bile was carried out by disc diffusion method by measuring zone of inhibition in mm, tetracycline was used as standard [15]. The activity of test and standard were screened against two gram positive such as *Bordetella pertusis, Staphylococcus aureus,* and two gram negative like *Eschericia coli* and *Vibrio cholerae* bacterial strains. Freshly prepared agar media was sterilized by autoclaving at 121ºC (15 lb/sq inch)

for 15 minutes. The sterile molten media was then aseptically transferred into four sterile petri dishes and kept in room temperature. Two milliliter of previously inoculated nutrient broth was then poured in each of the petridishes aseptically. The broth was then spread uniformly throughout the entire media. In each of the inoculated petridish, filter paper (Whatman No.1) disc of 6 mm diameter soaked well with the standard drug solution (Tetracycline) and with the crude samples of bile were accordingly placed. The plates were kept undisturbed for at least two hours at room temperature to allow diffusion of the solution properly in the nutrient media. After incubation of the plate at 37±1ºC for 24 hrs, the diameter of zone of inhibition surrounding each of the discs was measured with the help of zone reader. The results were obtained as mean of three observations.

Antidiabetic activity

Antidiabetic activity of bile of carp was performed in streptozotocin (STZ) induced diabetic mice. Diabetes was induced in rats by single intraperitoneal (i.p.) injection of STZ (150 mg/kg b.w.) in normal saline [16]. After a week, animals with marked hyperglycemia (fasting blood glucose 200-300 mg%) were used for the study. The blood glucose level was checked by adopting orthotoluidine method [17].

Diabetic animals were divided into eight groups, each containing six animals. The bile samples at 100% concentration (crude bile content) and 50% diluted bile sample (Bile content:water for injection = 1:1) were administered intraperitoneally and orally in respective groups of mice in the dose of 200 mg/kg body weight. The blood glucose levels for each animal of each group were estimated after 4, 6, 24 and 48 hours of administration.

Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Turkey test. Results are expressed as mean ± SEM (n=3 for antimicrobial study, n=6 for antidiabetic evaluation) in each group. P values <0.05 were considered significant.

RESULTS

The physicochemical parameters such as color, pH, specific gravity, R_f value were recorded for bile content of *Catla catla* and *Labeo rohita* and tabulated in Table 1. The pH values of both bile content was found acidic and R_f values obtained from TLC studies are the evidence of at least 3 major components present in the bile content of both fishes.

The bile content of both the fishes was containing both organic and inorganic matter including pigment. The results of qualitative tests also revealed that bile content of both the fishes contain cholesterol, bile salt of deoxycholic acid and cholic acid, bile pigment, fatty acid. Chloride, sodium, potassium, calcium, phosphate, nitrate, carbonate and bicarbonate are present in bile as inorganic constituents. The stability of the bile content for both the fishes was not appreciable. The stability testing data showed that bile content of both the fishes were stable upto 4 h at 0ºC and 2 h at 4ºC, 1 h at room temperature and 37.7ºC. At 50ºC bile content was not stable at all (Table 2).

The antibacterial activity data of crude bile material was given in Table 3, result suggests that bile content were active against *B. pertusis, S. aureus, E. coli* and *V. cholerae* but not at par to standard tetracycline for both the cases.

A remarkable antidiabetic activity was observed in streptozotocin induced hyperglycemic mice after both intraperitoneal and oral administration of bile samples. The results were given in Table 4. Intraperitoneal injection of bile content of *Catla catla* in 100% concentration showed average reduction in diabetes by 64.88%, 76.94% after 6 h and 24 h respectively, while oral administration showed 39.00%, 53.47%, 74.62% reduction of diabetes after 6, 24 and 48 h respectively. The intraperitoneal and oral administration of bile content in 50% concentration after 48 h of administration showed maximum 75.56% and 72.57% diabetes reduction respectively. But it was also observed that crude bile material killed mice after 24 hrs of intraperitoneal administration, so that the reading after 48 hrs has not taken in this case.

A remarkable antidiabetic activity was also observed for bile content of *Labeo rohita* but the mortality of animals was higher. Bile content in 100% concentration through intraperitoneal route was showed 77.82% and 78.67% reduction of diabetes after 4 h and 6 h respectively, while administration through oral route caused reduction of diabetes by 57.27% and 75.98% after 6 h and 24 h respectively. It was also noticed that all the animals were died after 6 h and 24 h in case of 100% bile content administration through intraperitoneal and oral route respectively. Intraperitoneal administration of bile content (50% concentration) showed 50.08% and 74.98% reduction in diabetes after 4 h and 6 h, while 40.83%, 61.43% and 76.89% reduction in diabetes observed after 6 h, 24 h and 48 h of oral administration of 50% bile content of *Labeo rohita* respectively. All the animals were died after 24 h after intraperitoneal of 50% bile of *Labeo rohita*, but all the animals were alive till 48 h after oral administration.

DISCUSSION

The traditional people are depends on natural resources from time immemorial for their health care needs. This knowledge usually transmitted orally from generation to generation without any written document. Therefore research based on such information could lead the discovery of new treatment strategy or drug molecules. It was also well proved that bile acids in human confer gut mucosal protection against bacteria [18]. Several researches also proved that several fish part like epidermal mucus of cat fish, fish oil may exert antimicrobial activity [19, 20]. The results of this study indicate that that the bile of these fishes contains one or several components with antibacterial activity. During antidiabetic study it was found that in maximum cases individual animal was showing drowsiness before death, though no abnormality was found. Among fresh water fishes two major Indian carps *Catal catla* and *Labeo rohita*, are most widely cultivated in Tripura, India. Stability of bile content was not appreciable. It can also be concluded that fresh bile content of *Catla catla* and *Labeo rohita* showed remarkable antidiabetic activity along with certain level of antibacterial activity. However, more investigation is necessary to confirm the antidiabetic and antibacterial activity of bile content of *Catla catla* and *Labeo rohita* and to purify and characterize the active components.

Table 1: Physicochemical parameter of crude bile content of *Catla catla* and *Labeo rohita*

Crude bile content	Colour	pH	Specific gravity	*Rf value*		
				Spot A	Spot B	*Spot C*
Catla catla	Reddish brown	5.7	1.09	0.09[$]	0.16[$]	*0.33*[$]
				0.23[#]	0.46[#]	*0.89*[#]
Labeo rohita	Reddish brown	5.8	1.10	0.08[$]	0.19[$]	*0.29*[$]
				0.30[#]	*0.48*[#]	*0.92*[#]

Slica gel G used as stationary phase to determine Rf value.
Moblie phase [$]Ethyl acetate : Water (1:1); [#] Butanol : Water : Dioxan (2:1:2)

Table 2: Absorbance of freshly collected bile content of *Catla catla* and *Labeo rohita* at different temperature and at different time interval at their λmax

Temperature	Time (hour)	Absorbance	
		***Catla catla* (λmax = 580 nn)**	***Labeo rohita* (λmax = 470 nn)**
0ºC	1	0.63	0.08
	2	0.63	0.08
	4	0.63	0.08
	24	0.19	0.04
4ºC	1	0.63	0.08
	2	0.63	0.08
	4	0.24	0.05
	24	0.19	0.04
37.7ºC	1	0.63	0.08
	2	0.19	0.06
	4	0.11	0.05
	24	0.04	0.03
50ºC	1	0.19	0.07
	2	0.09	0.05
	4	0.08	0.02
	24	0.04	0.01
Room temperature (27-28ºC)	0	0.63	0.08
	1	0.63	0.06
	2	0.21	0.05
	4	0.13	0.03
	24	0.09	0.01

Table 3: Antibacterial activity data bile content of *Catla catla* and *Labeo rohita*

Crude material/Drug	Zone of inhibition in mm against bacterial strains (Mean±SEM)			
	B. pertussis	***S. aureus***	***E. coli***	***V. cholerae***
Bile (*Catla catla*)	13±0.9	9±0.8	13±1.1	16±1.3
Bile (*Labeo rohita*)	14±1.2	12±0.9	12±1.0	9±1.4
Tetracycline[#]	23±1.1	24±1.4	24±1.2	23±1.0

DMSO used as solvent. Values are expressed as mean±S.E.M (n=6)
[#]Tetracycline used as standard at a concentration of 200 µg/mL.

Table 4. Antidiabetic activity of crude bile content of *Catla catla* and *Labeo rohita*

Sample and concentration	Route of administration	Observation after time in hour	Percentage of Diabetes reduced (Mean± SEM) (n=6)
Bile of *Catla catla* (100% concentration)	Intraperitoneal	4	54.08 ± 0.329
		6	64.88 ± 0.529
		24	76.94 ± 0.235
	Oral	4	28.10 ± 0.222
		6	39.00 ± 0.422
		24	53.47 ± 0.447
		48	74.62 ± 0.202
Bile of *Catla catla* (50% concentration)	Intraperitoneal	4	45.06 ± 0.911
		6	57.26 ± 1.021
		24	65.76 ± 0.753
	Oral	48	75.56 ± 0.433
		4	25.02 ± 0.721
		6	32.42 ± 1.092
		24	44.81 ± 0.810
		48	72.57 ± 0.284
Bile of *Labeo rohita* (100% concentration)	Intraperitoneal	4	77.82 ± 0.49
		6	78.67 ± 0.33
	Oral	4	50.41 ± 0.39
		6	57.27 ± 0.44
		24	75.98 ± 0.22
Bile of *Labeo rohita* (50% concentration)	Intraperitoneal	4	45.93 ± 1.02
		6	50.08 ± 0.75
		24	74.98 ± 1.11
	Oral	4	37.40 ± 0.56
		6	40.83 ± 0.79
		24	61.43 ± 0.28

Values are expressed as mean±S.E.M (n=6)

CONCLUSION

This study suggested that bile content of both fresh water carps can be utilized with its medicinal importance particularly in bacterial infection and diabetic condition, though further research obviously may be necessary for dose fixation and identification of active constituents.

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