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In vivo Effects of Apigeninisolated from *Jatropha gossypifolia* plant on the Biochemical Profile of Fish

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Abstract: Laboratory evaluation was made to develop an eco-friendly and effective herbalpiscicide. Piscicidal activity of compound Apigenin extracted from the plant *Jatropha gossypifolia*against the freshwater predatory fish *Channa punctatus* was time and dose dependent. Exposure of sub lethal doses of Apigenin caused significant ($P<0.05$) time and dose dependent anddecreasement in the level of total protein, nucleic acids (DNA and RNA), glycogen andincreasement in Amino acid level and activity of enzymeproteases in both liver and muscle tissues of fish *Channa punctatus*. Withdrawal study shows that there is a significant recovery in all the above biochemical parameters, in both tissues of fish after the 7th day of the withdrawal of treatment, which supports the view that the herbal product is safe to be used as piscicide for the control of freshwater target animal as well as predatory and weed fishes of freshwater culture ponds.

Key words: Apigenin · *Jatropha gossypifolia* · Piscicide · Predatory Fishes · Trash Fishes

INTRODUCTION

In recent times, use of medicinal plants as effective alternatives to synthetic pesticides and fertilizers has gained more importance because they are more effective, less expensive, biodegradable and safe for environment, than synthetic pesticides [1, 2]. Plant extracts are referred to as Botanicals and when poison to fish is called piscicides [3]. Several plants belonging to different families, which posses a number of compounds as, tannins, alkaloids, saponins, di- and tri-terpenoids etc. havehigh pesticidal activity and used in freshwater bodies to control harmful snails, disease causing insects, such as mosquito larvae and weed fishes [4-11]. Some fishes are uneconomic, small in size that naturally occur and accidently introduced in fish culture pond along with carp spawn and predate the fry and fingerling stage of carp fishes, called weed fishes.Air breathing predatory fish species, such as *Channa punctatus, Channa marulius*; etc. causes special problems because they may survive in moist burrows, even when ponds are drained [12]. The presence of predatory and weed fishes in fish culture pond pose the serious problem for fisherman in India, so removal of unwanted fish population from the culture pond is necessary before the seeds of cultured carps areadded.

*Jatropha gossypifolia*is a common medicinal plant in India, methanol, acetone and diethyl ether extracts of its latexhaving high molluscicidal, piscicidal and insecticidalactivities[13-16]. The acute toxicity of latex powder of *Jatropha gossypifolia*and Apigeninhave beenreported [17], but without knowing their mode of action and sub lethal effect, we cannot recommend direct use ofthis compound in culture ponds for control of predatory and weed fishes.

The present investigation deals with the biochemical effect of sub lethal doses of Apigenin extracted from *Jatropha gossypifolia* leaves on the level of total protein, total free amino acids, nucleic acids (DNA and RNA), glycogen and activity of enzyme proteases, in the liver and muscle tissues of *Channa punctatus*.

MATERIALS AND METHODS

Collection of Experimental Animals: Freshwater fish *Channa punctatus* (63.86±1.50 g body weight; 18.37±1.34

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cm. in total length)were collected from Ramgarh Lake of Gorakhpur (U.P) India, the collected fish were maintain in glass aquarium containing 100L of de-chlorinated tap water for acclimatization to laboratory condition for 21 days. The aquarium water was aerated continuously by electronic aerator. The proper artificial fish food supply is given for natural health maintenance of fishes. The dead fishes were removed from the aquarium to avoid any type of contamination.

Collection of Plant Materials: The leaves of plant *Jatropha gossypifolia* were collected from Botanical Garden of DDU Gorakhpur University, Gorakhpur and plant was identified by Prof, S.K. Singh,Taxonomist, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, (U. P.) India, where the voucher specimen is deposited.

Extraction of Apigenin from Leaf: The Apigenin was isolated from the leaf of*Jatropha gossypifolia* by the method of Subramanian *et al.* [18]. The leaves of*Jatropha gossypifolia* were washed properly by tap water and cut the leaves by scissors and then dried in shady place and further dried in an incubator at about 35°C temperature, the dried leaves were powdered by electric Grinder. About 50g powder of leaves was subjected in Soxhlet extraction unit with about 250-300 ml ethyl alcohol for about 72hrs at 30-40°C when extraction was completed then filtered and a little amount of crude yellow powder was obtained. After addition of NaOH and HCl,Apigenin was obtained, which was crystallized by methanol.Apigenin extracted from leaves of*Jatropha gossypifolia* were confirmed by UV spectra data of Dordevic*eet al.* [19].The chemical structure of Apigenin is illustrated in (Fig. 1).

Biochemical Analysis: Protein levels were estimated bythe method ofLowry *et al.* [2] using bovine serum albumin as standard. Tissue were removed and homogenise(50 mg/mL, w/v) were prepared in10% TCA.Tissuewere homogenised for 5 minutes using an electric tissuehomogenizer and centrifuged at 6000g for 20 minutes. Values have been expressed as µg/mg of tissue.

Total free amino acids were estimated using the method of Spices[21]. Homogenates (10 mg/ml, w/v) were prepared in 96% ethanol in an electric tissue homogenizer for 5 minutes and centrifuged at8000 g for 20 minutes and supernatant was used for amino acid estimation. Freeamino acids have been expressed as µg/mg of tissue.

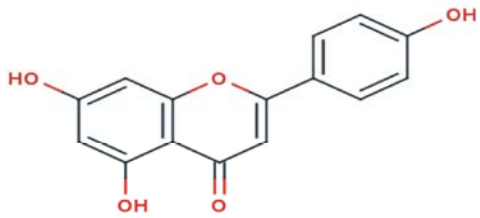

Fig.1 Chemical structure of Apigenin

Estimation ofnucleic acid (DNA and RNA) was performed, by the methods of Schneider[22] using diphenylamineand Orcinol reagents, respectively. Homogenates (100 mg/ml, w/v) were prepared in 5% TCA at 90°C by electric homogenizer for 5minutes and centrifuged at 5000 g for 20 minutes and supernatant was used for estimation. Both DNA and RNA have been expressed asmg/gm tissue.

Glycogen was estimated by the Anthrone method of Van der Vies, [23]. Homogenates (100 mg/ml, w/v) were prepared in cold 5% TCA and 1.0 ml of filtrate was used for assay. Result has been expressed as mg glycogen/g of tissue.

Proteaswas measuredaccording to methodof Moore and Stein [24]. Homogenates (50 mg/ml, w/v) were prepared in cold distilled water and centrifuged at 1000 g for 15 minutes and supernatant was kept for enzyme assay. The enzyme activity was expressed in µmoles of tyrosine equivalent/mg/protein/hr.

Withdrawal Experiment: In order to see the effect of withdrawal treatment, the fishes were exposed for 96 hrs to 72.6mg/l. Half of the animals were sacrificed and the activities of all the above biochemical parameters were measured in the liver as well as muscle tissues of fishes. The other half were transferred to freshwater, which was changed every 24 hrs for the next seven days. Control animals were held insimilar condition without any treatment. Following this, all the above biochemical parameters were measured in the liver and muscle tissues.

RESULTS

Exposure to sub lethal doses ofApigenin after 24hrs, caused significant alterations in nitrogenous as well as carbohydrate metabolism of the fish *C. punctatus*in both

liver and muscle tissues (Tables 1 and 2). Total protein, nucleic acid (DNA and RNA) and Glycogen levels were significantly reduced (P<0.05), while free amino acid level and activity of enzymeprotease were significantly enhanced (P<0.05) in liver and muscle tissues after exposure to sub lethal doses.After 24hrs exposure, data shows that theprotein level was reduced to 81% in liver cells and 85% in muscles cells with respect to control at 40% of LC_{50}, likewise at 80% of LC_{50} the similar trend was observed,the protein level was reduced to 74% in liver cell and 80% in muscles cells with respect to control. Amino acid is the unit of protein, when protein breakdown into amino acid then the level of amino acid increases, same trend was found in this case. Amino acid was increased by 110% in live, 109% in muscles cell at 40% of LC_{50} and same like 116% in liver cells, 112% in muscles cells with respect to control at 80% of LC_{50}. DNA was reduced to 80% in liver and 87% in muscles cells at 40% of LC_{50},same things was found like 80% in liver and 84% in muscles cells with respect of control at 80% LC_{50}. RNA was alsoreduced to 78% in liver cells and 80% in muscles cells at 40% of LC_{50} and in the case 80% of LC_{50} was 74% and 77% in liver and muscles cells respectively with respect to control. Glycogen level was reduced to 65% in liver cells and 70% in muscles cells at40% of LC_{50} and 55%, 62% in liver and muscles cells respectively at 80% of LC_{50} with respect to control.In protease the level was enhanced by 121% in liver cells and 114% in muscles cells at 40% of LC_{50} and in 80% of LC_{50} 133% and 131% in liver and muscles cellsrespectively with respect to control (Table 1).

Exposure of 40% of LC_{50} for 96h of compound Apigenin, the protein level was reduced to 84% in liver cells and 88% in muscles cellsafter 96h and 74% and 84% in liver and muscles cells respectively at 80% of LC_{50} with respect to control after 96h. Amino acid was enhanced by 113% in liver and 112% in muscles cells at 40% of LC_{50} and 119%, 116% in liver and muscles cells respectively at 80% of LC_{50} with respect to control. DNA depleted by 88% in liver and 90% in muscles cells at 40% of LC_{50} and also decreases by 84%, 87% in liver and muscles cellsrespectively at 80% of LC_{50} with respect to control, In same manner RNA also decreases by 81% in liver and 83% in muscles cells at 40% of LC_{50} and 77%, 80% in liver and muscles cells respectively at 80% of LC_{50} with respect to control. Glycogen level was reduced to 68% in liver cells and 73% in muscles cell at40% of LC_{50} and 65%, 72% in liver and muscles respectively at 80% of LC_{50} with respect to control. In the case of protease the level was enhanced by 106% in liver and108% in muscles cells at 40% of LC_{50} and 114%, 109% in liver and muscles cells respectively with respect to control.Table (2) also shows that, on the 7th day after termination of treatment with Apigenin, there was nearly complete recovery in the levels of protein, amino acid, nucleic acids, glycogen and activity of enzyme protease (Table 2).

Table 1: Changes in total protein, total free amino acids, nucleic acids (DNA and RNA), Proteases and glycogen levels in different tissues of fish *Channa punctatus*after exposure to 40% and 80% ofLC_{50} (24hrs) of active compound Apigeninextracted from leaf of*Jatropha gossypifolia*.

Parameters	Tissue	Control	40% LC_{50} (24h)	80% LC_{50} (24h)
Protein (µg/mg)	Liver	19.88±0.110 (100)	16.10±0.109* (81%)	14.71±0.29* (74%)
	Muscle	16.99±0.200 (100)	14.44±0.168* (85)	13.55±0.24* (80)
Amino acid (µg/mg)	Liver	20.84±0.182(100)	22.94±0.24*(110)	24.17±0.12*(116)
	Muscle	29.62±0.226(100)	32.28±0.32*(109)	33.17±2.00*(112)
DNA(mg/mg)	Liver	20.52±0.116(100)	17.44±0.10*(85)	25.62±1.33*(80)
	Muscle	13.32±0.129(100)	11.58±0.32*(87)	11.18±0.36*(84)
RNA (mg/mg)	Liver	29.42±0.138100)	22.94±0.196*(78)	21.77±0..12*(74)
	Muscle	35.50±0.187(100)	28.40±0.134*(80)	27.33±0.28*(77)
Glycogen mg/g)	Liver	25.17±0.330 (100)	16.36±0.253* (65)	13.84±1.66* (55)
	Muscle	11.96±0.257 (100)	8.37±0.375* (70)	7.41±0.31* (62)
Protease	Liver	0.65±0.021 (100)	0.54±.031 (100)	0.79±0.031(121)
(m tyrosine/mg protein/hr)	Muscle	0.62±0.014 (114)	0.87±0.024* (133)	0.71±0.032* (131)

Values are mean ± SE of six replicates.

Values in parentheses are % of control value.

Data were analyzed through student't' test.

* Significant (P< 0.05), when treated groups were compared with controls.

Table 2: Changes in total protein, total free amino acids, nucleic acids (DNA and RNA), proteases and glycogen levels in different tissues of fish *Channa punctatus*after exposure to 40% and 80% of LC_{50} (96hrs) of active compound Apigenin extracted from leaf of *Jatropha gossypifolia*.

Parameters	Tissue	Control	40% LC_{50} (96hrs) (36.38mg/l)	80% LC_{50} (96hrs) (72.6mg/l)	Recovery after 144hrs of withdrawal
Protein (µg/mg)	Liver	19.88±0.110 (100)	16.10±0.109* (84%)	14.71±0.29* (74%)	18.36±0.34)$^+$ (92)
	Muscle	16.99±0.200 (100)	14.95±0.168* (88)	14.27±0.24* (84)	15.60±0.21$^+$ (91)
Amino acid (µg/mg)	Liver	20.84±0.182(100)	23.54±0.24*(113)	24.79±0.12*(119)	21.36±0.31$^+$(102)
	Muscle	29.62±0.226 (100)	33.17±0.32* (112)	34.35±2.00* (116)	32.68±0.10$^+$ (110)
DNA (µg/mg)	Liver	20.52±0.116 (100)	18.14±0.10* (88)	17.23±1.33* (84)	199.96±0.24$^+$ (97)
	Muscle	13.32±0.129 (100)	11.98±0.32* (90)	11.58±0.36* (87)	12.68±0.32$^+$ (95)
RNA (µg/mg)	Liver	29.42±0.138 (100)	23.84±0.196* (81)	22.65±0..12* (77)	28.46±0.21$^+$ (97)
	Muscle	35.50±0.187 (100)	29.46±0.134* (83)	28.40±0.28* (80)	33.34±0.68$^+$ (93)
Glycogen (mg/g)	Liver	25.17±0.330 (100)	17.11±0.253* (68)	14.59±1.66* (58)	23.96±0.53$^+$ (95)
	Muscle	11.96±0.257 (100)	8.73±0.375* (73)	7.77±0.31* (65)	10.68±0.54$^+$ (89)
Protease	Liver	16.36±0.24 (100)	15.96±0.34 (100)	17.46±0.42 (106)	17.36±0.32 (108)
(m tyrosine/mg protein/h)	Muscle	18.77±0.12 (114)	17.46±0.21 (109)	17.68±0.24$^+$ (108)	16.76±0.45$^+$ (105)

Details are given in Table 1

+ Significant ($P< 0.05$), when withdrawal groups were compared with treated groups.

DISCUSSION

Behavioral response of fishes exposed to sub lethal concentrations of the compound Apigenin showed that after exposure, fishes were stressed. During stress, fish need more energy to detoxify toxicants and to overcome stress. Since fishes have very little carbohydrate, protein is used to meet the increased energy demand. Proteins are mainly involved in the architecture of the cells, which is the chief source of nitrogenous substancemetabolism. Thus the depletion of protein level in liver and muscles tissues may have been due to their degradation and possible utilization for metabolic purposes. Increases in free amino acids level were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis [25]. It is also attributed to lesser use of amino acids [26] and their involvement in the maintenance of an acid-base balance [27]. Natarajan [28] suggested that stress conditions induce elevation in the transamination pathway. The decrease in total protein level and increases in total free amino acids level in both tissues suggest the high protein hydrolytic activity due to elevation of protease activity. Inhibition of DNA synthesisthus, might affect both protein as well as amino acids level, by decreasing the level of RNA in protein synthesis machinery. The results of this study suggest that the extracted compound Apigenin is a potent inhibitor of DNA synthesis, which in turn results in the reduction of RNA level. Mahendru [29] suggested thatanti-AChE compounds attack many types of enzymes responsible for normal metabolic pathway. Thus, it is possible that extracted compoundsApigenin might have inhibited the enzymes necessary for DNA synthesis. In the case of Carbohydrates,it is the primary and immediate sources of energy. In stress condition, glycolysis takes place to fulfill the energy demand. Several reports are available on the effect of muscular exercise on liver glycogen energy reserves in fish, which get depleted [30, 31]. Liver glycogen levelwas depleted during acute hypoxia or physical disturbances in fish [32]. Pesticides are also inhibited energy production by suppressing aerobic oxidation of carbohydrate leading to energy crisis in animals [33]. Carbohydrate metabolism is broadly divided into two segments- (1) Anaerobic segment of glycolysis (Embden- Meyerhof-parnas pathway) in which break down of glucose occurs (2) Aerobic segment of glycolysis, which consists of oxidation of pyruvate to acetyl co-A to be utilized through citric acid cycle. The end product of glycolysis under anaerobic condition in tissue is lactic acid, whereas the pyruvate level in tissue can be taken as a measure of aerobic condition of tissue depending on the availability of molecular oxygen. The level of tissue lactate content acts as an index of anaerobiosis, which might be beneficial for animals to tolerate hypoxic condition [34] under pesticide exposure condition. In the case of liver and muscle, both aerobic and anaerobicconditions are likely to operate depending on availability of molecular oxygen and other physiological needs imposed by other factors. Increases of lactate content were accompanied by a decrease in pyruvate content in all tissues. The decrease in liver and muscle pyruvate levels andincrease in lactate content suggest a shift towards anaerobiosis as a consequence of hypoxia, created under pesticides toxic

impact leading to respiratory distress [35] and Siva Prasad Rao [36]. The decrease in pyruvate level may be due to its conversion to lactate or due to its mobilization to form amino acids, lipids, triglycerides and glycogen synthesis in addition to its role as a detoxification factor in ammonia toxicity [37]. Student't'test were applied for locating significant differences [38].We therefore believe that the active compound Apigeninpresent in the plant *Jatropha gossypifolia* may eventually be of great value for the control of aquatic target organisms as well as predatory and weed fishes.

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