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Research Article

Evaluation of toxicity of Dichlorvos (Nuvan) to fresh water fish *Anabas testudineus* and possible modulation by crude aqueous extract of *Andrographis paniculata*: A preliminary investigation

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ABSTRACT

Dichlorvos (DV) is widely used pesticide due to their low persistence in aquatic ecosystem but it affects the non-target organisms as well. In the present investigation aqueous leaf extract of *Andrographis paniculata* (AP) was tested for its possible modulatory changes in *Anabas testudineus* against dichlorvos induced toxicity by taking into consideration several biomarkers of toxicity viz. activity of enzymes in blood and liver (Alkaline phosphatase ALP, Acid phosphatase ACP and gama-glutamyl transferase, GGT) haematological variables at 3 exposure times i.e. 7,15 and 45 days. Fish were divided Group I: normal control without any treatment, Group II: Dichlorvos treatment at a dose of 0.39 ppm (µg L^{-1}) which is $1/5^{th}$ of LC_{50} value of 96 hr, Group III. Dichlorvos treatment at a dose of 0.39 ppm plus aqueous AP treatment (83.32 ppm which is $1/3^{rd}$ of IC_{50} value), Group IV, Fish treated with only 83.32 ppm of AP ($1/3^{rd}$ of IC_{50} value). Analysis of the data reveals that treatment with AP extract reduced the activity of ACP, ALP and GGT at all the three exposure times namely 7, 15 and 45 days ($p<0.001$), similarly there was a significant reduction in creatinine, urea and blood glucose content in fish treated with *Andrographis paniculata* when compared to only dichlorvos treated group ($p<0.05$ through $p<0.001$). A similar trend was also observed when comparing the haematological profiles ($p<0.001$). On analysis of the leaves it was found to contain several phytoconstituents through UV-vis spectrophotometric, gas chromatography and mass spectrophotometry and biochemical studies. Collectively the results suggest that treatment with aqueous *Andrographis paniculata* extracts could favorably modulate the toxicological response in fish induced by dichlorvos and therefore represent a promising alternative for sustainable aquaculture practices.

INTRODUCTION

Pesticides are widely used in agriculture and indiscriminate use of pesticides resulted in unintentional poisoning of the environment. Pesticides are fairly common in many South Asian countries where rural population works in close proximity to these compounds and hence, they are relatively more vulnerable. Pesticides pose a hazard to public health and environmental integrity in the biodiversity-rich Asia-Pacific. The changeable and stimulating food security situation together with rapid and unplanned urbanization progress resulted in increased and indiscriminate use of pesticides in agricultural as well as domestic sectors. Lack of consciousness and education coupled with indifferent attitude also contributed to indiscriminate use of pesticides. Pesticides belong to organophosphates, carbonates and organochlorine groups. Modern agriculture techniques contributed to enhance crop production, but it also extensively contaminated aquatic environment. Organophosphates are preferred during recent years because they persist in the environment for brief period and are biodegradable (Oruç *et al.*, 2006, 2007; Ye *et al.*, 2010). Among them Dichlorvos, which belongs to organophosphate group gained prominent consideration by the agriculturalist as an insecticide (USEPA, 1994, 2007). Further, veterinarians use it as anti-helminthic agent on horses, pigs, dogs, on the other hand it is also used by fishermen to eradicate ectoparasites of fish (USPEA, 1994; Varo *et al.*, 2003; Deka and Mahanta 2015). These pesticides accumulate in aquatic ecosystem by direct

application where they induce undesirable effects in non-target organism such as fish, and crustaceans (Fulton and Key, 2001; Nwani *et al.,* 2017). Dichlorvos exert its effect in fish by inhibiting acetylcholine esterase activity which results in an increase in the level of acetylcholine at the site of synaptic cleft and produces anomalies like sudden changes in swimming, impaired feeding, excess mucous secretion, distorted spatial orientation and impaired reproductive behavior (Howard 1991; Bretaud *et al.,* 2000; London *et al.,* 2005; Okoroiwu and Iwara 2018).

Aquaculture both marine and inland capture fisheries is a still a growing industry to meet the global aquatic food requirements (FAO, 2016). Practice of using chemotherapeutic agents and synthetic antibiotics however provide profit to the business but its indiscriminate use is unjustifiable as they cause accumulation of residues at various trophic levels and are hazardous to public health (WHO 2017). In such a scenario there is an increasing demand of aquatic foods which are devoid of chemicals and synthetic drugs. Hence, it is necessary that a natural or eco-friendly tactic be developed to confirm sustainability of aquaculture.

Fish serves as an excellent ecotoxicological model for assessment of risk and testing toxicity and indicator of contamination of aquatic bodies (El-Amrani *et al.,* 2012). In this regard *Anabas testudineus* is an important fish species in Indian subcontinent and preferred for its delicacy and nutritional value. Further, this species is perennial and they can acclimatize in any condition.

Medicinal herbal extracts stand out as probable substitutes to chemicals in aquaculture as they contain several phytoconstituents which comprises of biologically active metabolites with diverse benefits (Bulfon et al 2013; Zanuzzo et al 2015; Yilmaz and Ergün 2018; Yilmaz 2019a, b). Numerous works have been conducted worldwide by using herbal extracts in aquaculture such as caffeic acid was used against *Aeromonas veronii* as an anti-oxidant and to improve immunological functions along with liver gene expression in *Oreochromis niloticus* (Yilmaz 2019). Güllü *et al.,* 2016, reported that dietary administration of *Pimenta dioca* modulated haematological, immunological variables and biochemical responses in *Oreochromis niloticus*. It was reported by others that rutin isolated from *Toona sinensis* has strong antioxidant and anti-stress activity in crustaceans which modulated favourably the enzymological, immunological and haematological variables to stress in *Litopenaeus vannamei* caused by *Vibrio alginolyticus* (Hsieh *et al.,* 2011). Improved values of haematological, biochemical and immunological parameters were also found in shrimp fed with a diet that consisted of methanol extracts of *Cynodon dactylon, Aegle marmelos, Tinospora cordifolia, Picrorhiza kurroa* and *Eclipta alba* (Citarasu *et al.,* 2006). Further, it was reported that crude extracts of *Aloe vera* enhanced antioxidant enzymes and reduced stress in fish (Gabriel 2015a).

In this context *Andrographis paniculata* (AP) commonly known as Kalmegh in Bengali is a plant of wide importance due to its pharmacological properties and grown in Asian subcontinent and traditionally used against various ailments and reported to have antioxidant (Singh *et al.,* 2001; Adedapo *et al.,* 2015; Mussard *et al.,* 2019), anti-inflammatory (Shen *et al.,* 2000; Sheeja *et al.,* 2006), hepatoprotective (Handa and Sharma 1990; Kapil *et al.,* 1993; Chandler *et al.,* 1995), cardiovascular (Zhang and Tan 2000; Tan and Zhang 2004), anticancer (Ajayakumar *et al.,* 2004) and revert sexual dysfunction (Akbarsha and Murugain 2000) properties. Wang and Zhao (1994) reported that extracts of AP have potentials to avert constriction of blood vessel and increases blood clotting time. Further, Dua *et al.,* 2004 reported antimalarial activity of xanthones isolated from the roots of AP. Effective application of plant extracts in aquaculture is limited due to lack of adequate knowledge of plants, functions, extraction procedure and effective dose of treatment.

Hence, the present investigation was conducted to find out whether AP can modulate favorably the changes induced by dichlorvos in chronically treated fish (*Anabas testudineus*) at three exposure times, viz. at days 7, 15, and 45 against suitable controls by taking into consideration several enzymological, haematological parameters.

MATERIALS AND METHODS

Collection and Acclimatization of fish samples

Specimens of *A. testudineus* (mean weight 12.05±0.85g) were procured from local market, and were allowed to acclimatize for 15 days in glass aquarium containing 5 litres of dechlorinated tap water under laboratory environment (temperature 29.0°C±1.45°C, pH 7.3±0.08, DO 5.24±0.18mg/L, Hardness 215.0±5.32 mg/L, Total alkalinity 176.0±4.32) with constant aeration and natural photoperiod condition. Water was renewed every 4th day. The fish were fed a balanced diet containing 30% crude protein during acclimatization and feeding was discontinued 24 hours prior to sacrifice of the test animals.

Preparation of the leaf extract

Plants were gathered from the adjacent villages of West Midnapore district, West Bengal and recognized. A voucher sample was deposited in Botany department (V-1250 MDC/2015) for record. The leaves so collected were sundried and then (250 g) extracted in 50% alcohol (the ratio being plant material to solvent 1:10m/v). The extraction process was carried out with constant stirring for 24 hours at 50°C. The extract was evaporated to dryness with the help of a Soxhlet apparatus and then kept at 4°C until required. The yield of the extract was 13.22%, computed by the equation =W1/W2x100, where (W1=the weight of extract after evaporation of solvent, W2=the original weight taken of the dry leaf material, respectively).

Preliminary phytochemical Screening

The phytochemical screening of AP leaves for alkaloids, tannins, reducing sugars, glycosides, flavonoids, carbohydrates and steroids was analysed by routine procedures (Biswas et al 2019).

Total antioxidant assay and phenol content

The antioxidant activity of the leaf extract of AP and their standard was assessed based on the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by the modified method of Nooman *et al.,* 2008. In brief, various working solutions of test extracts were made in methanol and butylated hydroxytoluene (BHT) was used as standard/reference in a 1-100 µg/ml

solution and also 0.002% of DPPH was made (dissolved in methanol). To 1 ml of sample and standard solution, 1 ml of 0.002% DPPH solution was mixed and thoroughly shaken and kept in darkness for 30 minutes. Then O.D. (optical density) was taken at 517 nm using (UV-1800 Shimadzu) spectrophotometer. Optical density was documented and the % inhibition calculated using the formula. % age inhibition=A-B/A*100 where A is the OD of the blank and B is the OD of the sample. Total phenolics of the extracts were determined following the method of Sadasivam and Manikam (2008) with slight modifications.

UV-vis analysis of leaf

One g of AP leaf powder was kept overnight with 30 ml of Millipore grade water with constant stirring and then filtered. An aliquot of the filtered sample was scanned using UV-visible Spectrophotometer (Shimadzu, UV-1800), at a range of 320-740 nm (scanning speed-medium, and slit width 1), to detect the characteristic wavelength of the leaf extract.

GC/MS analysis of aerial parts

GC/MS analysis of AP leaf extract was conducted as described elsewhere (Biswas et al 2019). The Gas chromatograph was coupled to a mass spectrophotometer which was equipped with a fused capillary column, Model No: Agilent 190915-433 (HP-5MS, 0.25mm×30m ×0.25µm). For GC/MS detection the carrier gas was helium with a persistent flow rate of the sample at 1mL/min and ionization energy of 69.9eV being utilized. The sample volume injected was 5µl in GC grade ethanol at an average velocity of 37cm/sec. The temperature of the column was initially set to 5 minutes at 50°C then it was programmed to 280°C. The total GC running time was 28 minutes.

Toxicity tests of dichlorvos and AP determination of sublethal concentration

Acute toxicity bioassay procedure was directed according to the standard methods (APHA 1998). LC_{50} of dichlorvos to *A. testudineus* was estimated by exposing six groups of fish (5 per group) to differing concentration of dichlorvos with the experiment was repeated twice to obtain the LC_{50}-96 hour value of the pesticide. The LC_{50} value of DV was obtained 1.95 ppm (µg L^{-1}) for *A. testudineus* following the Probit analysis method as previously reported by Finney's (1971). Similarly, IC50 value of AP was 83.32 ppm of AP ($1/3^{rd}$ of IC_{50} value) was also obtained.

The specimens of fish were now exposed to one concentration of dichlorvos in a semi-static system. The exposure was carried out for 7, 15 and 45 days respectively. They were divided into following sets.

Group I: normal control without any treatment, Group II: Dichlorvos treatment at a dose of 0.39 ppm (µg L^{-1}) which is $1/5^{th}$ of LC_{50} value of 96 hr, Group III. Dichlorvos treatment at a dose of 0.39 ppm plus aqueous AP treatment (83.32 ppm which is $1/3^{rd}$ of IC_{50} value), Group IV, Fish treated with only 83.32 ppm of AP ($1/3^{rd}$ of IC_{50} value).

Treatment and control were conducted in triplicate and on the sampling day i.e. 7, 15 and 45 days. Blood was collected from the caudal vein with heparinized syringe and the whole blood was utilized for the analysis of the haematological profiles. Serum isolated from blood was used for the analysis of the biomarkers of toxicity such as ALP (alkaline phosphatase), ACP (acid phosphatase), GGT (Gama glutamyl transferase). Further amount of creatinine, urea, and blood glucose were also determined. Activities of different enzymes stated above were also analysed from liver samples.

Hematological analysis

The whole blood with anticoagulant trisodium citrate was subjected to Hematoanalyzer (SB22 Plus VET, India) for analysis of WBC, RBC, Haemoglobin, HCT, PLT, MCV, MCH and MCHC.

Enzymatic analysis

For analysis of alkaline phosphatase (ALP) and acid phosphatase (ACP) the method of Kind (1954) was followed, Briefly, 50µl of serum was added to 1500µl of substrate which consisted of phenyl phosphate, phenol and 4 amino-antipyrine. The orange red colour complex so formed was read at 510 nm against a suitable blank.

For analysis of the gamma glutamyl transferase (GGT) the method of Szasz (1976) was followed. In brief 1000µl of working reagent consisting of gamma glutamyl-p-nitroanilide (GPNA) and glycylglycine was mixed with 100 µl of serum and the rate of increase in absorbance was noted at 405nm against a suitable blank. Estimation of Urea, total cholesterol and HDL cholesterol was followed according to manufacturer's instruction (Span diagnostics Ltd.).

Behavioral bioassay

The behavioral bioassay was carried out in static type experimental design following the protocol of US-EPA-660/3-75-009, the fish were introduced into the aquaria and allowed to acclimatize for 72 hours after that the toxicant was added to the water. Fishes of both treated and control sets were noted for immobilization, excess mucous secretion, opercular movement, scale loss, changes in the colouration of the body. Dead fish were removed from the aquaria periodically.

Statistical Analysis

At least three experiments were conducted and the data expressed as mean (±SE) and analyzed by one-way analysis of variance (ANOVA) and p-value less than 0.001 was considered statistically significant.

RESULTS

The phytochemical screening of the leaves of the AP revealed presence of various compounds which have been listed in Table 1. Further, the total antioxidant activity of aqueous leaf extract was quite high when compared to standard which has been summarized in Table 2 while the total phenolic content was of aqueous leaf extract was 81.22 mg/100 gm of dried leaves.

Table 1: Preliminary phytochemical screening of AP leaf extracts

Chemical compounds	Alcoholic Leaf extracts of AP
Flavonoids	+++
Saponins	+
Alkaloids	+++
Reducing sugars	++
Tannin	++
Carbohydrate	++
Steroids	+
Glycosides	+

AP-*Andrographis paniculata*, +: Minute, ++: Less abundant, +++: Much abundant

Table 2: Total antioxidant activity of leaf extract of AP [a] *p<0.05,* [b] *p<0.01,* [c] *p<0.001*

	% free radical activity (IC_{50} values)		
Sample	**10mg**	**20mg**	**30mg**
Standard(BHT)	50.88±0.78	52.14±0.98	55.16±0.75
AP	80.76±1.25[c]	81.87±1.11[b]	83.80±0.02[b]

UV-vis spectrophotometric analysis of the leaf revealed 22 peaks which are listed below (Table 3). Peaks at 487 and 483nm revels presence of flavonoids and its derivatives.

Table3: UV-vis spectrophotometric data of aqueous leaf extract

No.	Wavelength nm.	Abs.
1	659.5	0.077
2	532.5	0.019
3	520	0.083
4	512	0.096
5	505	0.091
6	496.5	0.103
7	487	0.11
8	483	0.185
9	444	0.254
10	437	0.057
11	426	0.407
12	414	0.206
13	550.5	0.017
14	523.5	0.018
15	507	0.086
16	503	0.081
17	490.5	0.086
18	485	0.021
19	479	0.085
20	440.5	0.031
21	418.5	0.202
22	342	0.142

On analysis of the extract through GC-MS it revealed that there are 15 peaks some of which have narrow to huge percentage peak area and retention time (Table 4). Some of the important phytoconstituents include phytol, Isosteviol methyl ester, coumatetralyl isomer, Androstan-17-one etc. some of which have biological properties.

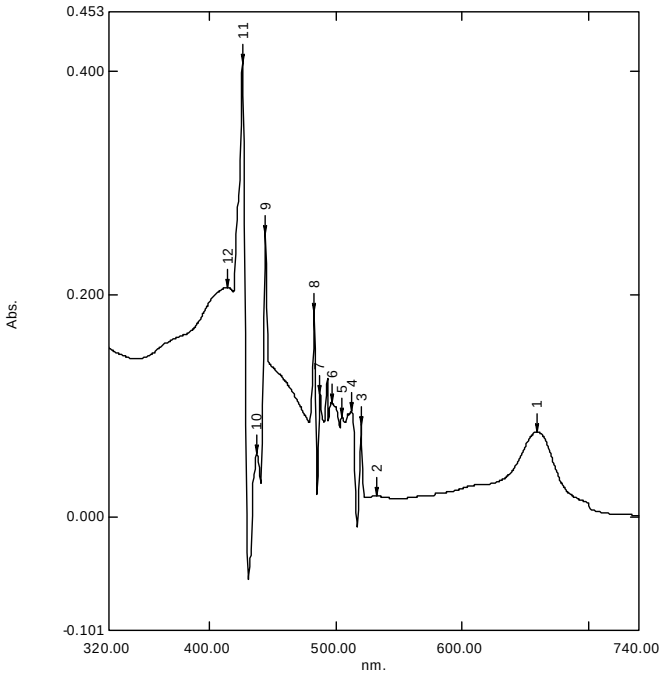

Fig. 1: UV-Vis spectrum of the aqueous leaf extract of AP

Table 4: Compounds identified in the leaf extract of *Andrographis paniculata* by GC-MS study

No	RT	Name of the compound	Mol formula	Mol.wt	Peak area %
1	11.03	Diethyl phthalate	$C_{12}H_{14}O_4$	222	0.66
2	13.98	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	5.71
3	14.28	Phthalic acid	$C_{23}H_{36}O_4$	376	1.08
4	14.52	Phytol acetate	$C_{22}H_{42}O_2$	338	1.66
5	16.03	Coumatetralyl isomer-2ME	$C_{20}H_{18}O_3$	306	0.51
6	17.67	Phytol	$C_{20}H_{40}O$	296	14.09
7	20.42	Docosenoic acid	$C_{22}H_{42}O_2$	338	0.31
8	21.45	Butyl 6,9,12,15-octadecatetraenoate	$C_{22}H_{36}O_2$	332	0.46
9	21.92	Octadecane	$C_{26}H_{54}$	366	0.90
10	23.78	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	1.28
11	24.80	Eicosane	$C_{26}H_{54}$	366	0.38
12	26.04	8,11,14-Eicosatrienoic acid	$C_{20}H_{34}O_2$	306	0.57
13	28.09	Isosteviol methyl ester	$C_{21}H_{32}O_3$	332	0.29
14	32.39	Androstan-17-one	$C_{21}H_{34}O_2$	318	25.77
15	33.28	3-Oxatricyclo (20.8.0.0(7,16) triaconta-1(22),7(16), 9,13,23,29-hexane	$C_{29}H_{42}O_2$	406	46.32

On careful examination of the activity of alkaline phosphatase in serum it revealed that there was a steady increase in activity from 7 to 45 days exposure interval in the only DV treated fish when compared to DV+AP, control and only AP treated fish. When the data of only DV and DV+AP groups were compared it revealed that ALP decreased significantly at all the three exposure intervals

which was statistically significant ($p<0.01$ though $p<0.001$, Fig. 2A, df=5). However, there was slight increase in activities of ALP in only AP treated fish when compared to only control. A similar trend was also noticed when the liver ALP was examined but the activity was much higher when compared to serum (Fig. 2B).

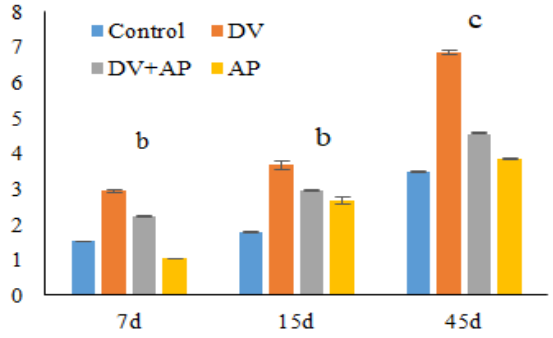

Fig. 2a: Activity of Alkaline phosphatase in serum at different fixation intervals (KA units) [a] $p<0.05$, [b] $p<0.01$, [c]$p<0.001$

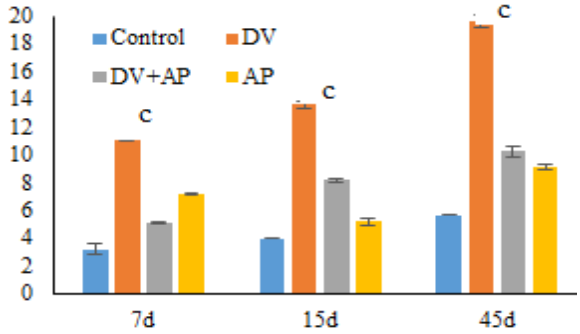

Fig. 2b: Activity of Alkaline phosphatase in liver at different fixation intervals (KA units) [a] $p<0.05$, [b] $p<0.01$, [c]$p<0.001$

Similarly, when the activities of serum acid phosphatase (ACP) were compared it revealed that its activity rose steadily from 7 to 45 days of DV treatment. This activity was much more when compared to control and only AP treated group. However, administration of AP to DV treated groups resulted a decrease in activity of the ACP ($p<0.05$ though $p<0.001$, Fig. 3A, df=5). When we analyzed the results of ACP activity in liver it also showed that the activity was highest in the DV treated series at all the exposure times i.e.7, 15 and 45 days when compared to only AP, control and DV+AP treated series. However, there was a considerable reduction in the activity of ACP in liver in the DV+AP treated group ($p<0.001$, Fig. 3B, df=5).

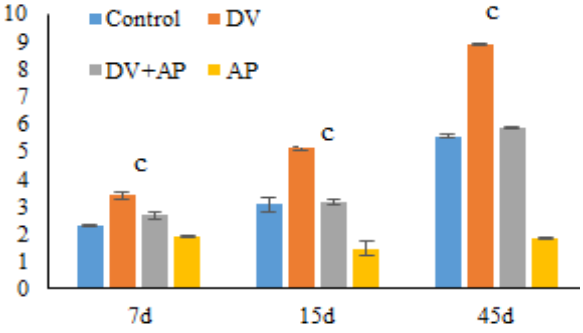

Fig. 3a: Activity of Acid phosphatase in serum at different fixation intervals (KA units) [a] $p<0.05$, [b] $p<0.01$, [c]$p<0.001$

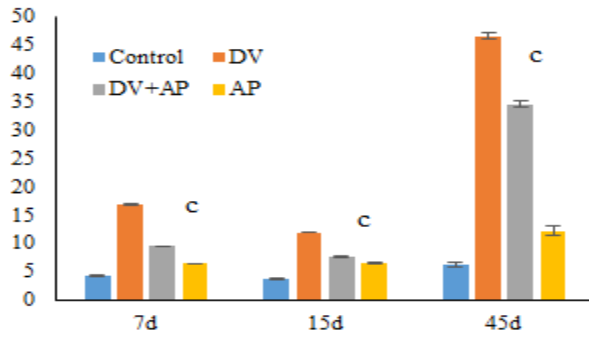

Fig. 3b: Activity of Acid phosphatase in liver at different fixation intervals (KA units) [a] $p<0.05$, [b] $p<0.01$, [c]$p<0.001$

GGT is one of the most sensitive biomarkers of the hepatobiliary system and elevated serum GGT levels are indicative of disease of hepatobiliary system. When we compared the activity of serum GGT between different groups, it revealed that the activity was appreciably more in the DV treated series when compared to control, AP treated and DV+AP treated group. AP treatment along with DV administration resulted in decrease activity of GGT at all the three exposure times ($p<0.001$, Fig. 4A, df=5). We also noted a similar trend when we compared the values of the activity of GGT in liver samples ($p<0.001$, Fig. 4B) however, it may be noted that there was slight increase in GGT activity in liver at all the three fixation intervals in the group that was administered only AP though it was not statistically significant.

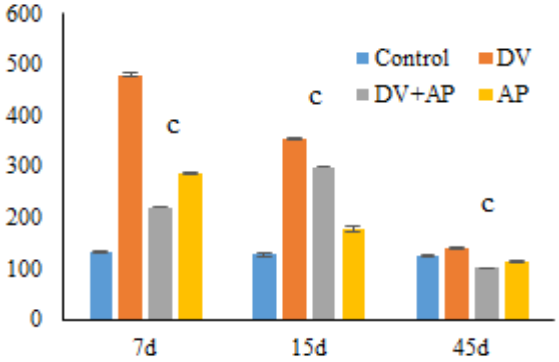

Fig. 4a: Activity of Gamma glutamyl transferase in serum at different fixation intervals (U/L) [a] $p<0.05$, [b] $p<0.01$, [c]$p<0.001$

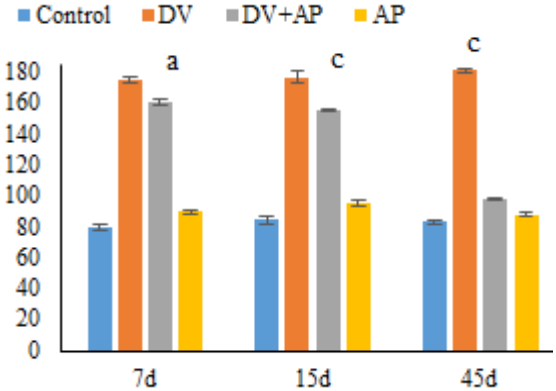

Fig. 4b: Activity of Gamma glutamyl transferase in liver at different fixation intervals (U/L) [a] $p<0.05$, [b] $p<0.01$, [c]$p<0.001$

Creatinine is the catabolite product of creatinine phosphate which is used by muscles, on careful examination of the content of creatinine in serum it was found that it was

more in the DV treated fish when compared to DV+AP group, only AP group and control at all exposure time. When the data of only DV treated and DV+AP treated were compared it was found that creatinine content was appreciably low in DV+AP treated group ($p<0.001$, Fig. 5A, df=5). We also observed that liver creatinine content showed a similar trend ($p<0.05$ through $p<0.001$, Fig. 5B). The creatinine content was quiet high in only AP treated group in both serum (45 days) and liver (7 days) at some exposure intervals.

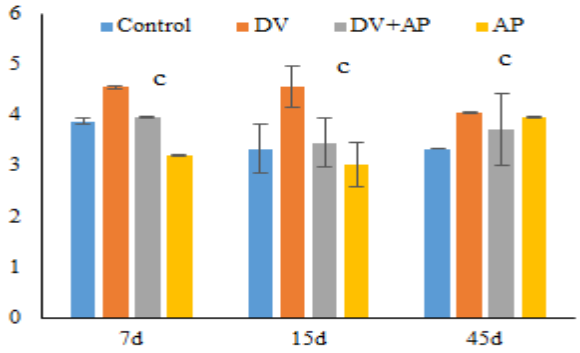

Fig. 5a: Serum Creatinine content (mg/dL) at different fixation intervals [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$

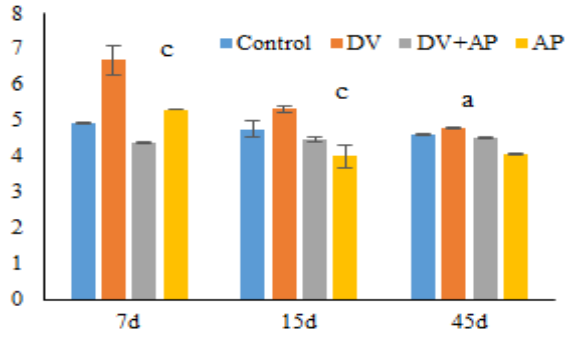

Fig. 5b: Liver Creatinine content (mg/dL) at different fixation intervals [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$

In fish, urea is produced in liver and excreted through gills and on careful examination of the data on urea we found that the urea content is more in DV treated group when compared to control, only AP treated and DV+AP treated groups at all fixation intervals. When DV and DV+AP treated groups were compared it revealed that AP treatment significantly reduced the urea content in DV treated group. This trend was also noticed while analyzing the urea content of liver ($p<0.001$, Fig. 6A, B, df=5) However, we also noticed that urea content was also slightly more in only AP treated groups when compared to untreated control fish at certain exposure times in case of serum and liver.

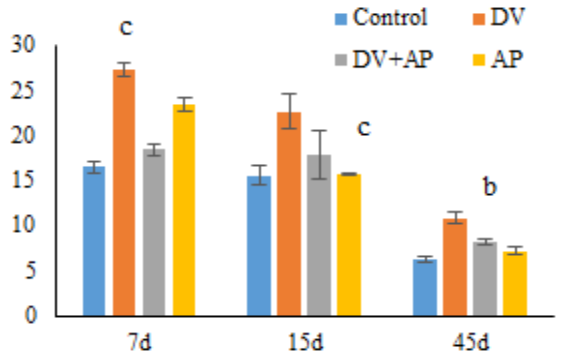

Fig. 6a: Serum urea content in (mg/100mL) at different fixation intervals [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$

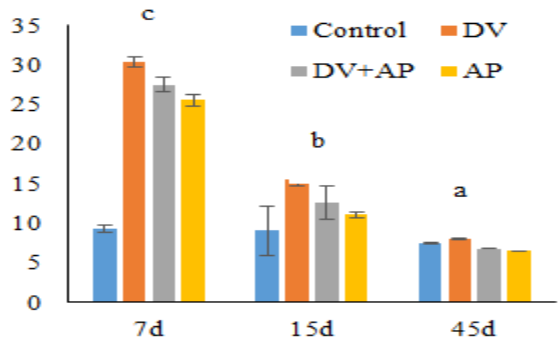

Fig. 6b: Liver urea content in (mg/100 mL) at different fixation intervals [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$

Our results showed that the blood glucose levels were expressively increased in DV treated fish on 7th day while it reduced considerably on 15th and 45th day when compared to control. However, we found that blood glucose levels were in DV+AP and only AP treated groups were more initially at 7 day which decreased further at next two fixation intervals (Fig. 7).

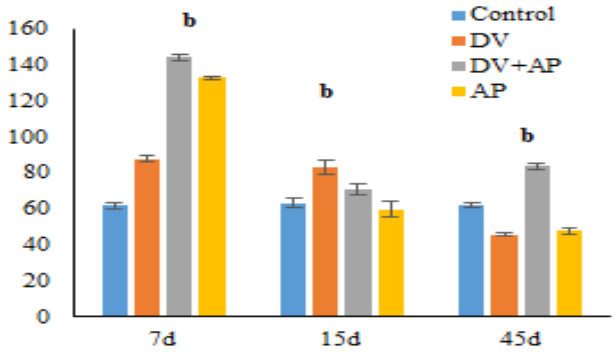

Fig. 7: Serum blood glucose (mg/dL) at different fixation [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$

On careful analysis of the data of the various haematological profiles it was evident that WBC count was lowest in the control series when compared to only DV treated and only AP treated groups at all exposure times. In the only DV treated group WBC count was highest at all the three exposure times of 7,15 and 45 days. A similar trend was also noted in the RBC count except at 7 day exposure interval where it was highest DV+AP and only AP treated group of fish (Table 5), The haemoglobin content was highest in DV+AP treated group at 7 and 15 days. HCT% decreased from 7 to 45 days in all the groups, it was highest in only DV treated group at 7 and 45 days when compared to control, AP and DV+AP treated groups ($p<0.001$, Table 5). On the other hand, PLT was appreciably low in DV treated group when compared to control. HCT% was more in DV+AP treated group at all the three fixation intervals. MCV value was lowest in the control group at all the three fixation intervals, it was highest in the DV+AP treated group when compared to all the other three groups. When the data of MCV of DP+AP was compared to only DV treated group it was statistically significant at all the three fixation intervals ($p<0.05$ though $p<0.001$, Table 5, df=5). The MCH value was highest in the DV+AP treated groups when compared to only DV treated group ($p<0.001$) and also when compared to other groups. Meanwhile this trend was also evident when we compared the values of MCHC ($p<0.001$, Table 5).

Table 5: Haematological profiles of fish of different treated and control groups at the three fixation intervals (df=5; [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$)

Parameters	Control	DV	DV+AP	AP
		7 days		
WBC (109/L)	0.36±0.20	1.32±0.07	2.06±0.11	1.50±0.08
RBC (1012/L)	0.44±0.16	1.61±0.14	1.89±0.22n	1.81±0.22
HGB (g/L)	10.00±0.29	53.21±4.05	66.50±3.33[a]	60.12±2.89
HCT (%)	5.81±0.34	22.72±3.09	26.95±6.67[a]	25.05±2.07
PLT (109/L)	12.12±0.89	5.01±0.36	23.51±2.98[c]	15.20±.86
MCV (fL)	131.81±7.05	140.2±4.87	142.59±6.77[n]	138.39±8.98
MCH (Picogram)	227.27±5.87	329.19±6.12	351.85±4.98[a]	331.49±6.71
MCHC (g/dL)	172.41±3.59	234.11±5.01	244.89±4.06[a]	239.52±3.05
		15 days		
WBC (109/L)	0.46±0.11	1.47±0.78	1.66±0.32	0.55±0.14
RBC (10^{12}/L)	0.55±0.18	1.71±0.06	1.03±0.05	0.53±0.07
HGB (g/L)	16.11±2.01	33.63±3.08	44.11±2.77[a]	17.66±0.89
HCT (%)	7.51±2.33	25.66±0.89	27.33±3.08[n]	8.66±0.46
PLT (109/L)	11.0±0.25	7.01±2.01	23.44±0.45[b]	12.07±0.35
MCV (fL)	133.66±5.68	147.66±7.09	149.33±6.33[n]	142.44±5.08
MCH (Picogram)	276.31±11.07	333.44±6.88	367.18±4.42[a]	277.11±6.97
MCHC (g/dL)	188.37±9.02	227.66±6.78	242.11±5.03[a]	199.32±9.07
		45 days		
WBC (109/L)	0.24±0.01	0.54±0.07	0.53±0.06n	0.26±0.03
RBC (10^{12}/L)	0.75±0.03	0.85±0.12	0.58±0.22[a]	0.39±0.16
HGB (g/L)	24.22±2.01	28.01±3.43	23.47±3.86	12.84±1.03
HCT (%)	10.41±2.01	11.94±0.11	8.72±0.98[a]	5.41±1.02
PLT (109/L)	13.11±1.02	19.47±1.02	37.84±3.06[b]	10.21±0.98
MCV (fL)	138.66±3.65	140.01±6.55	150.44±7.44[n]	138.46±3.09
MCH (Picogram)	320.44±4.02	329.41±6.04	396.55±0.97[b]	307.64±6.99
MCHC (g/dL)	230.76±1.06	235.29±5.02	264.36±7.48[b]	222.18±8.02

DISCUSSION

Organophosphates are the most commonly used pesticides in South East Asia and replaced organochlorines because of their effectiveness and non-persistence in the environment. As a result of their wide use and easy availability, they are involved in a greater number of poisoning not only to target animals but also to non-target species. In the present investigation it was evident through study of the different enzymes that the toxicity steadily increased in all the fixation intervals in fish that was treated with only DV. Increase in WBCs count occurs with extreme stress response since WBCs play a major role during insult of chemicals by stimulating the hemopoietic tissues and the immune system by producing antibodies and chemical substances which acts as a defense system (Jayaprakash and Shettu 2013). In the present investigation we encountered that WBC count was more in only DV treated and DV+AP treated groups initially at 7 day and 15 day which came down drastically at 45day exposure times. WBCs plays a pivotal role in the immune defense mechanism and respond instantly to the changes in medium due to the toxicant. In the current investigation we found that initially at 7 day there was a huge increase in WBC count in only DV treated group which slowly decreased as the investigation progressed to 45 day. We also encountered increase in haematocrit, RBC and haemoglobin content in only DV and DV+AP and only AP treated groups of fish initially at 7 and 15 days which reduced at 45 days. Similar results were also obtained by Svobodova et al (1994, 2008), they also found that increase in haematocrit, RBC count and haemoglobin in brook trout during 6 day and 21 days study on exposure to copper. In our investigation the increase in haemoglobin is due to increase in RBC count so it can be said that there might be some interference with regard to oxidation process in fish initially at 7 day, it might be that DV and AP might interfere with oxygen carrying capacity of blood and hence under stressed condition it stimulates erythropoietic process and hence increasing the haemoglobin content at 7 day which came down drastically in the next two exposure time. Urea is produced by liver and excreted by gills, increased urea levels can occur in liver impairment, infections and impaired kidney functions. The present elevation of serum urea and liver urea in only DV treated groups of fish at all the three exposure times (7, 15 and 45 days) may be due to the gill dysfunction which led to disturbance in diffusion of urea between blood and water, similar reports were also reported by other investigators (Suchismita 2013; Alkaladi *et al.,* 2015). In the present study we found that there was an elevation in serum urea and creatinine in only DV treated group which was significantly reduced both in the serum and liver after treatment with AP. Blood glucose has been shown to be a good and sensitive indicator of environmental pollution (Silbergeld 1974; Firat et al 2011), the increase in the value of blood glucose in DV treated group compared to controls group and only AP treated group indicated that dichlorvos might generated more glucose to produce energy to counter stress. Use of plant-derived products represent a hopeful tactic complementary to synthetic chemical, as they provide a useful source of active secondary metabolites which are biologically active, being at the same time easily available, inexpensive and biocompatible (Mohamad & Abasali 2010).

The use of medicinal plants in aquaculture has fascinated a lot of attention globally and has become a theme of active scientific investigations (Chakraborty and Hancz 2011; Harikrishnan *et al.,* 2011b). It was reported by others that the response of fish after the administration of plants and plant products was usually dose-dependent and therefore more manifestations were seen at higher concentrations. In the present investigation we choose the dose of 83.32 ppm of AP extract because this dose gave better results in our pilot studies and which is in line with other investigation conducted by other investigators (Sakai 1999; Prasad and Mukthiraj 2011; Bulfon *et al.,* 2013). Unselective use of pesticides and various antibiotics leads to accumulation of residues in non-target organisms, fish tissues and these are potentially dangerous to consumers as they deposit in various trophic levels of food chain (Casida 2009, WHO 2017). In the present investigation, we found there was an increase in ALP, ACP and GGT in only DV treated groups of fish at all the three exposure times and AP treatment reduced their activity quite significantly. Elevation of ACP may be attributed to destruction of the membranes of lysosome and release of ACP. Phosphatases catalyze the release of phosphoric acid from certain mono-phosphoric esters, a reaction of considerable importance.

Acid and alkaline phosphatases have been directly implicated to cellular damage and toxicity whose activity was favorably modulated after AP treatment which was found in the present study. γ-GGT on the other hand is an established marker for toxicological studies which catalyzes transfer of gamma-glutamyl functional groups from glutathione to acceptor and plays a critical role in synthesis and degradation of glutathione and detoxification process. In the present study the activity of γ-GGT was considerably reduced after treatment with AP extracts. The detoxifying activity of AP aqueous extracts might be attributed to several phytoconstituents present in the extract such as phytol, androstane-17-one. Rattanachaikunsopon and Phumkhachorn (2009b) reported the prophylactic result of the extracts of AP to counter infection of *Streptococcus agalactiae* in Nile tilapia. There were reports of diverse toxicity of dichlorvos and chlorpyrifos in endangered *Tor putitora* and detrimental effects of dichlorvos on AMPK signaling pathways in brain of chicken were also reported by some investigators (Kunwar et al 2020; Xiao *et al* 2020). Further, Oladokun et al 2020 reported deleterious genotoxic effects on administration of sub-lethal concentration of dichlorvos on *Clarius gariepinus*. Various groups have also stated the ill effects of dichlorvos in various mammalian species. Moura et al 2020 reported the risk of haematological malignancies in humans exposed to organophosphate pesticides for longer duration. Sub-chronic inhalation of dichlorvos has the ability to induce ototoxicity in rats were also reported (Reis *et al* 2020).

Shoot extracts of AP contains various phytochemicals such as diterpenoids, flavonoid and diterpene glycosides, lactones, flavonoids, the roots and shoot plant are also used as a folkloric therapy for different ailments in various Asian and European countries (Zhou *et al.,* 2008; Hossain *et al.,* 2014). A variety of systematic evaluations completed by plant extracts and their bioactive compounds have revealed that they have pharmacological properties. Few scientists also described the capability of *Ipomoea aquatica* extracts in reducing hepatotoxicity and oxidative stress in rats and detoxifying properties of *Polygonum equisetiforme* against dichlorvos induced oxidative stress and neurotoxicity in clam *Ruditapes decussatus* (Salahshoor et al 2020; El Ayari et al 2020). We also found flavonoids, alkaloids and phenols were present in the extracts in considerable amount. It is likely that the compounds present in aqueous AP extract especially the flavonoids and alkaloids might have the capability to detoxify DV induced toxicity which may be due to phenolic hydroxyl groups attached to the ring structures of flavonoids. The ability of flavonoids to scavenge hydroxyl radicals, superoxide anion radicals, and lipid peroxyl radicals is important for preventing oxidative cell membrane damage, damage to proteins and DNA, thereby making it a good contender for health-promoting functions *in vivo*. It is well known that extracts of plants have antioxidant potential and probably due to this they are used as medicine since time immemorial (Khalaf *et al* 2008). Phytol, is an aromatic, acyclic unsaturated diterpene alcohol, widely present in Vitamin E, K, and tocopherols which possess good antioxidant potentials. Huge amount of phytol and steroid androstane-17-one were present (% age peak area was 25.77 and 46.32 respectively) as revealed in the GC-MS analysis of the extract.

CONCLUSION

Though the accurate mechanism of the modulatory action of AP leaf extract is not clarified but it can be safe to conclude that the collective actions of all the constituents might contribute cooperatively to their protective effects against DV toxicity. This further supports that antioxidant compounds present in the extracts of AP may act as potential candidates for preventing the toxicity in fish induced by DV. We urge others to confirm and refute our findings.

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