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COMPARATIVE STUDY OF SOME ANTIBIOTICS ON BIOCHEMICAL CHANGES IN PLASMA OF THE SHARPTOOTH CATFISH *CLARIAS GARIEPINUS.*

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ABSTRACT

Ciprofloxacin (15 mg/kg body weight), amoxycillin (50 mg/kg body weight) and ampicillin (80 mg/kg b. wt.) administered orally to male sharptooth catfish *Clarias gariepinus* 3 times, every 72 hours intervals. Plasma samples were taken for three successive days after the last dose the most important results were :

1) Plasma glucose levels were significantly elevated in fish administered ciprofloxacin and amoxycillin at the 3 days studied and ampicillin at the 3rd day. In contrast, hypoglycemia observed in the first two days in plasma of *C. gariepinus* administered ampicillin. 2) Significant hyperproteinaemia was observed in fish groups administered ~~both ciprofloxacin~~ and amoxycillin antibiotics during the three days studied. Ampicillin showed a significant highest value at the second day, while the values at both 1st and 3rd days showed significant lower values. 3) Administration of the three antibiotics significantly raised the plasma urea in the three days studied except the 2nd and 3rd days in fish administrated amoxycillin and ampicillin respectively. Also, increased plasma concentrations of uric acid and creatinine in *C. gariepinus* administered the three antibiotics at the three days studied were recorded. 4) The AST and ALT activities in *C. gariepinus* administered both ciprofloxacin and amoxycillin antibiotics showed significant higher values at the 3rd day. The slope of rise of AST and ALT activities in both ciprofloxacin and amoxycillin were greater than that of ampicillin that is approximately similar to the control group. So, ciprofloxacin, amoxycillin followed

by ampicillin are effective on the liver functions in a decreasing order. This observation is clear in ALT than AST, which indicates that ALT is more sensitive to antibiotic administration. 5) Analysis of ALP activity in all groups of *C. gariepinus* administered with the three antibiotics in the three periods studied showed significant higher values. The administration of antibiotic may considered as stress factor in the fish even when it used in the prophylactic dose. Glucose and ALT, AST and ALP can used to evaluate the stress condition in fish especially in the farm condition.

INTRODUCTION

Clinical chemical analysis is a fundamental tool used in human and veterinary medicine to diagnose and predict the outcome of disease and to monitor the effect of therapeutic, nutritional, and environmental management. (Smith and Reynard, 1992) Little information about the effect of antibiotics on fish biochemistry and metabolism were detected, Abd El–Alim *et al.* (1998) studied the effect of ciprofloxacin and enrofloxacin on serum creatinine, AST, ALT and blood urea nitrogen in the Catfish *C. gariepinus*. Screening studies must be carried out to evalute the possible drawbacks of some obligatory used antibiotics, that will aid in therapeutic regimens of antibiotics and changing the outlook towards treatment measures in the future. This work done to detect the changes occur in fish plasma constituents when ciprofloxacin, amoxycillin and ampicillin are used in fish farms as prophylactics.

MATERIAL AND METHODS

Seventy two apparently healthy male catfish sharptooth (*Clarias gariepinus*) with approximately as uniform in length (24.5-29.5cm) were collected from River Nile (El-Aiat area), Giza Goveronerate. All fish under experimentation were individually examined and recorded for freement from any skin lesions or furunculosis particularly basis of pectoral and pelvic fins as recorded by Foda (1973).

Fish were distributed into twelve glass aquria (150 X 50 X 75 cm). These aquaria were supplied with dechlorinated tape water with continous aeration. The water changed completely every 3 days and faeces and remainder of food syphoned every day. Fish were acclimated for 2 weeks. They fed on cooked and sterile chipped

chicken intestine once daily between 8 and 9 A.M. The average weight of sample fish was used to adjust feeding at 3 % and to calculate the doses of antibiotics. Feeding was stopped 24 hours before the administration of drugs or before sampling. Fish were grouped into 4 groups (3 aquaria / group). The first group represents the control group and given maize starch (mixed in maize starch powder "Schering-Cid. Pharmco. Industry". The three antibiotics given orally to the rest of the groups as filling material in gelatinous capsules was administered by finger into the stomach of *Clarias gariepinus* as follows: Group two: fish administered 15 mg Ciprofloxacin ($C_{17}H_{18}FN_3O_3$), (El-Ameriya Pharmaco Industry)/kg body weight (Novws *et al.*,1988). Group three: fish administered 80 mg Amoxycillin ($C_{16}H_{19}N_3O_5S$)(E.P.Co Pharmaco. Industry)/kg body weight according to Inglis *et al.* (1992). Group four: fish administered 50 mg Ampicillin ($C_{16}H_{19}N_3O_4S$) (E.P.Co Pharmaco. Industry)/kg body weight (Noga,1995). The capsules administered orally to the fish 3 times, every 72 hours intervals.

Plasma samples were taken for three successive days after the last dose from un-anesthetic fish for biochemical analysis (Hunn and Greer,1991).The following biochemical parameters were estimated: glucose (Trinder,1969); total protein (Henry, 1968); urea (Palton and Crouch,1977); uric acid (Haisman and Muller, 1977); and creatinine (Faulkner and King, 1976). The following enzymes were colorimetric determined: alanine aminotransferase, aspartate aminotransferase (Reitman and Frankel, 1957) and alkaline phosphatase (Eastman and Bixler, 1977) The results were statistically analysed (mean, standared deviation, t-test, ANOVA-test and LSD using the different formula mentioned by Hill, (1977).

RESULTS

The changes in plasma glucose level were summarized in Table (1). Significant ($P < 0.001$) lower values indicated in fish administrated ampicillin after the 1st and 2nd days compared with control group. After the 3rd day, significant ($P < 0.001$) higher value was recorded. Fish administrated ciprofloxacin and amoxicillin after the 1st, 2nd and 3rd days had significant ($P < 0.001$) higher values. Comparing the results between groups (using ANOVA and $LSD_{0.05}$) indicated that ciprofloxacin was the more effective one followed by the other two antibiotics. Using $LSD_{0.05}$ (group-time interaction) in

Table (3) demonstrated that ciprofloxacin was the more effective elevating plasma glucose level in fish followed by amoxicillin and ampicillin.

Significant ($P < 0.001$) lower values of plasma total protein (The results summarized in Table (2) observed in fish administrated ampicillin after the 1st and 3rd days compared with corresponding values of control group. Significant higher values indicated in fish administered ciprofloxacin after the 1st and 2nd days respectively. In Amoxicillin treated fish, total protein was close to level of control group after the 1st, 2nd and 3rd days. Comparing the results between groups, using both ANOVA and LSD test (Table 3), indicated that both ciprofloxacin and amoxicillin were more decreasing the plasma protein levels. The interaction between groups and time provided an indication that the effect of ampicillin appeared more pronounced followed by ciprofloxacin.

Fish administrated amoxicillin and ampicillin had significant ($P < 0.001$) lower values of plasma urea indicated after the 2nd and 3rd days respectively compared with control group. Using t-test, there was significant ($P < 0.001$) higher values indicated in fish administered ciprofloxacin after the 1st, 2nd and 3rd days, and amoxicillin after the 1st and 3rd days and ampicillin after the 1st day (Table 1). Comparing the results between groups indicated that ciprofloxacin was the effective one then the other two antibiotics. In relation to time, there was a significant value at the 3rd day. Table (3) demonstrated that amoxicillin appears its effect after the 2nd day followed by ciprofloxacin and ampicillin at the 3rd day.

Significant higher values of plasma uric acid were recorded in the three groups of fish administered the antibiotics at the three days except after the 1st day in fish administered ampicillin which showed no significant value. In relation to groups, ciprofloxacin showed significant difference to the other two antibiotics and the control group. Amoxicillin and ampicillin showed similarity but differ to the control group. In relation to time, the 3rd day is a common remark between the three days. The interaction between groups and times indicated that the highest effect of ciprofloxacin, amoxicillin and ampicillin (Table 3).

Fish administered ciprofloxacin and ampicillin had significant ($P < 0.001$) higher values of plasma creatinine level demonstrated after the three days studied. Also fish administered amoxicillin showed significant ($P < 0.001$) higher value after the 2nd day in contrast to the 1st day that showed significant ($P < 0.001$) lower value

while after the 3rd day, no significant value was detected. Comparing the results between groups indicated that there were significant differences between the three antibiotics and the ciprofloxacin elevate plasma creatinine levels, while amoxicillin was similar to the control. In relation to time, there was a significant indication, especially after the 1st day ; Table (3) demonstrated that ciprofloxacin was the most effective antibiotic especially after the 1st and 2nd days followed by ampicillin.

The results of Alanine aminotransferase were summarized in Table (2). Significant lower values indicated in fish administered ciprofloxacin, amoxicillin and ampicillin administrated fish after 1st day for the three antibiotics and 2nd and 3rd days for ampicillin. Significant higher values were indicated in fish administered ciprofloxacin after the 3rd day and amoxicillin after the 2nd and 3rd days. Comparing the results between groups indicated that amoxicillin was the highly significant increase alanine aminotransferase level in fish. Using ANOVA and $LSD_{0.05}$ and in relation to time, there was a significant record between the three days and the higher one after the 3rd day. Table (3) demonstrated that amoxicillin was the more effective one especially after the 3rd day followed by the other two antibiotics after the 3rd day.

Fish administered ciprofloxacin and ampicillin had significant lower values of aspartate aminotransferase were detected in the 2nd day, using t-test.(Table 2). Significant higher values were detected in fish administered ciprofloxacin after the 1st and 3rd days and amoxicillin after the 3rd day ($P < 0.001$). Comparing the results between groups using ANOVA and $LSD_{0.05}$ calculations indicated that ciprofloxacin was highly effective than the other two antibiotics. In relation to time, the effect of antibiotics was higher after the 2nd and 3rd days. The interaction between groups and time demonstrated that the effect of ciprofloxacin was the first one after the 3rd day followed by amoxicillin after the 2nd day and finally ampicillin after the 3rd day (Table 3).

Plasma ALP values showed significant ($P < 0.001$) higher values in all groups of fish administered the three antibiotics at the three days studied (Table 2). Comparison between groups demonstrated that ciprofloxacin, amoxicillin and ampicillin were significantly differ than the control group and arranged in a descending order. The interrelationship between groups and time

detected that strongest relation appeared in the ciprofloxacin group after the 2nd day (Table 3).

DISCUSSION

Blood biochemical values are not commonly used as a diagnostic tool in fish medicine, partly because of the lack of reference intervals for various fish species, and because changes in blood analyses associated with specific diseases and metabolic disorders are not well characterized. With sufficient background data, clinical biochemical analysis could be developed to detect metabolic disorders and sub lethal disease states affecting production efficiency.

Plasma glucose level in control *Clarias gariepinus* were approximately similar to the result of Mohamed (1981). Younis (1993) and Nounou *et al.* (1997) on serum and Shalabi *et al.* (2000) on plasma of *C. gariepinus* reported lower results. Siakpere (1985) recorded lower plasma glucose for *C. batrachus* and *C. isheriensis*. The significant elevated plasma glucose levels in fish administered ciprofloxacin and amoxicillin at the 3 days studied and ampicillin at the 3rd day compared with the respective controls indicated that antibiotics affects glucose dynamics in *Clarias gariepinus* to gain more energy to withstand and overcome the existing stress condition. Plasma or serum glucose levels is often used as an indicator of non-specific stress (Wedermeyer *et al.* 1981 ; Hunn and Greer, 1991), and is considered the most sensitive parameter in detecting sublethal stress responses (Fivelstad *et al.* 1995). West *et al.* (1994) mentioned that plasma glucose level was high in the exercised rainbow trout, but hyperglycemia was not related specifically to exercise. They said that while utilization of glucose in the whole animal was responsive to plasma glucose availability, estimated total skeletal white muscle (the largest homogeneous tissue mass in trout) utilization rate of glucose was low (less than 15 %) and was relatively insensitive to plasma glucose concentration. This situation emphasize that glucose availability was pertinent to tissues other than muscle and, further, that white muscle glycogen was re-synthesized essentially independently of glucose availability. Therefore, the largest tissue mass in the body utilized only a small portion of the glucose released into the circulation and Bever *et al.* (1981) suggested that glucose metabolism is involved largely with mucus production in fish (e.g. *Clarias gariepinus*) rather than energy provision. Gillis and Ballantyne (1996) further suggests that glucose concentration itself

may have been an important regulator of whole-body glucose disposal. This would be consistent with the generalization that glucose regulatory hormones are slow to respond to elevated glucose levels in teleosts (Hemre *et al.* 1991), but it is not known that regulatory characteristics are specifically involved with enhanced glucose production *in vivo*. On the other hand, Mesa *et al.* (1999) found that the chronic and progressive infection of juvenile chinook salmon, lead to depressed levels of plasma glucose indicating that the disease is stressful during the later stages. Depressed levels of plasma glucose in fish have been reported by others assessing the physiological effects of BKD (Iwama *et al.* 1986) and are probably due to excessive use of this energy substrate to help combat the infection (Mesa *et al.*, 1998). The hyperglycemia mentioned before in our study has been suggested to be due to either a shift in glucose from the tissues to the blood or to impairment of glucose mobilization. In contrast, hypoglycemia observed in the first two days in plasma of *C. gariepinus* administered ampicillin which seems to be due to a rapid use of glucose to overcome the stress of ampicillin used.

Proteins of blood serum are a fairly labile biochemical system, precisely reflecting the condition of the organism and changes taking place in it under the influence of internal and external factors. In the present study on *Clarias gariepinus*, plasma total protein of normal fish are approximately similar to Rizkalla, (1982); Nounou *et al.* (1997) and Shalabi *et al.* (2000) on serum and plasma of *C. gariepinus*. Soliman *et al.* (1991); Younis (1993) and Aly *et al.* (2000) recorded lower values of total proteins. Higher values of serum total proteins of *C. gariepinus* were recorded by Rizkalla (1988) and Abd El-Alim *et al.* (1998). Kori-Siakpere (1985) recorded lower values in serum of *C. isheriensis*. Significant hyper-proteinaemia was observed in fish groups administered both ciprofloxacin and amoxicillin antibiotics during the three days studied (except at the 3rd day in ciprofloxacin group in which total plasma proteins was insignificant high due to the high standard deviation of that day). A high serum total protein has been reported to be indicative of osmo-regulatory dysfunction, heam-dilution or tissue damage surrounding blood vessels (Wood *et al.*, 1983). Aly *et al.* (2000) revealed positive correlation between the total serum protein of vaccinated *C. gariepinus* with *Aeromonas hydrophilic* bacteria in comparison to the control once. This could be a response of the body

to the antigen. On the other hand, any parasitic, bacterial and viral disease to fish caused hypo-proteinemic effect (Byrne *et al*., 1998). Under the environmental and stress conditions to which fish were exposed in our investigation (6 individuals / aquarium and changes of water of aquarium every 3 days), plasma protein rose. This may be influenced by the high organic loads and bacterial counts characteristic of the high-density system of rearing. The organic material and bacterial loud could have induced a generalized immune response (i.e. increased globulin fraction of the total proteins) in these fish. This opinion is supported by Soliman *et al*. (1991) that the change in protein concentration in male fish was related to the synthesis of globulin fraction, while in females with accumulation of albumin in serum. Additionally, higher total proteins concentration could result from possible chronic gill inflammation in these fish (Hrubec *et al*., 1996). In contrast to our result, Abd El-Alim *et al*. (1998) found that ciprofloxacin and enrofloxacin caused significant decreases of serum total proteins of Nile catfish *C. gariepinus* in all exposure periods (5 days). They said that hypo-proteinaemia might be due to amino acids utilization as defiance against the pathogens and renal damage provoked by bacteria of drugs.

Ampicillin in our study (Table 1) showed a significant highest value which is higher than the control value at the second day, while the values at both 1st and 3rd days showed significant lower values compared to the control ones. These lower values make a contradictory way in our understanding, but theoretically, we can supposed that plasma protein decrease can result from haem-dilution, loss of proteins with urine following kidney damage, or by increased protein utilization without replenishment (Ozretic and Krajnovic-Ozretic, 1993).

Most teleost fish is obligate ammonioteles excreting the bulk 75 – 90 % of their waste nitrogen as ammonia (Mommsen and Walsh, 1992 and Hamdy and Poxton, 1993), together with only small amounts (5 - 15 %) of urea produced by uricolysis (Mommsen and Walsh, 1991 ; Wood, 1993). In turbot urea-N daily excretion rates represents 26 % of the sum of total ammonia nitrogen (TAN) and urea-N excretion (Dosdat *et al*., 1996). The control values of plasma urea in our study (Table 2) was similar result recorded by Mohamed (1981). Younis (1993) and Shalabi *et al*. (2000) recorded lower results. High plasma urea level s and urea excretion were found in an air –breathing cat fish *Heteropneustes fossilis* (Saha and Ratha, 1994 : Saha *et al*., 1988). Administration of the three antibiotics significantly

($P < 0.001$) raised the plasma urea in the three days studied excepted the 2nd and 3rd days in fish-administrated amoxicillin and ampicillin respectively (Table1). Abd El-Alim *et al.* (1998) showed that blood urea nitrogen level was significantly elevated up to 72 hrs of exposure to both ciprofloxacin and enrofloxacin which could be attributed to affection of the kidney along the course of the treatment. In mammals, urea is considered primarily a terminal metabolite. Walsh *et al.* (1990) found that urea excretion rates and plasma urea concentrations in toadfish were not of affected by antibiotic treatments. All the hypotheses assume that urea is synthesized in the liver and transported through blood to excretions site(s). to check whether other sites might be involved in urea production, Wood *et al.* (1995) found that urea excretion rates and plasma urea concentration in toad fish were not affected by antibiotic treatment. Also, in all teleosts studied, the gills appear to be the major site of ammonia and urea excretion (Wood, 1993). The gill lesions were apparently sufficient to hinder urea excretion. This was reflected in the general increase in serum urea values over the experimental period as compared with the control values (Gerundo *et al.*, 1991). More recently it has been shown in the laboratory that urea can be induced in gulf toadfish by confinement/crowding (Hopkins *et al.*, 1997). Confinement/crowding stress also causes a rapid (within 24 hr) several-fold activation of hepatic glutamine synthetase (GNS) (Walsh *et al.*, 1994). a central enzyme of ureo-genesis in toadfish (Mommsen and Walsh, 1991).

Large individual variations in plasma urea levels were found in the studies on rainbow trout (Wilkie and Wood, 1991) and Atlantic salmon (Knoph and Masoval, 1996). The large individual variations were thus not likely to be due to the feeding of the fish before sampling or the blood sampling site (Wood, 1993) but could possibly be explained in part by variation in the time of struggle and air exposure during blood sampling (Knoph and Masoval, 1996). So, the large individual variation of plasma urea level in Atlantic salmon together with the influence by factors such as water temperature and salinity, water ammonia and oxygen levels, fish size and density, and feeding (Person *et al.*, 1998) suggests that its use in diagnostic work on fish may be difficult (Knoph and Masoval, 1996) .

As well as urea, uric acid and creatinine are byproduct of protein catabolism. In fish, urea and uric acid reported to be voided through the gills and the kidney (Wright *et al.*, 1993). It penetrates

through simple aqueous pores rather than through a carrier (Yousef and Macey, 1989). Such passive excretion through "urea pores" has been observed in fish gills by Dosdat *et al.* (1996).

The control values of uric acid and creatinine (Table 1) are very higher than that recorded by Younis (1993) and Shalabi *et al.* (2000) on the same hish (*Clarias gariepinus*). Eckert and Randall (1983) reported that the high levels of uric acid are unusual and only minimal quantities are normally found in teleost plasma. Creatinine concentration in pike and Atlantic salmon sera shows significant correlation to water temperature. Sex and month as well as their interaction significantly influenced variation of serum creatinine level. (Lenhardt, 1992).

Increased plasma concentration of uric acid and creatinine in *Clarias gariepinus* administered the three antibiotics at the three days studied (table 1) can be attributed most probably to the degenerative changes of the kidney. In harmony with the present study. Abd El-Alim *et al.* (1998) showed that serum creatinine level of *C. gariepinus* was elevated up to 72 and 48 hrs. of exposure to ciprofloxacine and enrofloxacin respectively. This elevation of creatinine may be due to nephrotoxic effect of the drugs (Harrizon and Harrizon, 1986). Casillas *et al.* (1983) reported that increased blood level of creatinine was the most sensitive indicators of kidney damage in English sole. According to Holloway and Shoemaker (1993) the serum creatinine level may be an indicator of kidney dysfunction and important in predicting diseases in which the kidney is adversely affected.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) belong to the non-plasma specific enzymes which are localized within tissue cells of liver, heart, gills, kidneys. muscle and other organs (Gandet *et al.*, 1975) and in blood plasma they may give specific information about organ dysfunction (Wells *et al.*, 1986). When cell membranes are intact, these enzymes are present in blood in low concentrations, as the cell membranes are impermeable to enzymes when the cells are metabolizing normally (Hemre *et al*, 2000). Monitoring of liver enzymes leakage in the blood has proved to be a very useful tool in liver toxic studies (Salah El-Deen and Rogeps, 1993).

In the present investigation, the mean values of plasma AST and ALT activities in the control *Clarias gariepinus* for AST and ALT was same as that recorded by Shalabi *et al.* (2000). While Younis (1993) recorded higher AST and ALT values. Both Nounou *et al.* (1997) and Abd El-Alim *et al.* (1998) recorded higher values for

AST and lower values for ALT. The AST and ALT activities in *C. Gariepinus* administered both ciprofloxacin and amoxicillin antibiotics showed significant higher values at the 3rd day (table 3). The slope of rise of AST and ALT activities (i.e. the difference between the enzyme activity at the 3rd day minus enzyme activity at the 1st day) in both ciprofloxacin and amoxicillin were greater than that of ampicillin which is approximately similar to the control group. This observation is clear in ALT than AST which indicates that: (1) ALT is more sensitive to antibiotic administration, (2) ciprofloxacin, amoxicillin followed by ampicillin are effective on the liver functions in a decreasing order. Abd El-Alim *et al.* (1998) observed a slight increase in serum ALT and AST resulting from some liver damage produced by drugs and antibiotics (ciprofloxacine and enrofloxacin). The rise in the serum transaminses was correlated with the extent of celluar necrosis. AST and ALT activities are localized primarley in the cytoplasmic compartment, but a rise in these activities suggests lysis of hepatic origin. More specifically, the elevated AST values suggest cellualr destruction subsequent to pathologic steatosis (Michael *et al.* 1987) and during necrotic episodes following cell death in the liver (Heath, 1995). Carbis *et al.* (1996) mentioned that serum AST and ALT activity elevated in carp gavages with single and multiple doses of micro-cystins. Daily low dose exposure of carp to microcystins caused a small and transient increase in serum ALT activities.

To use serum or plasma ALP activity as a diagnostic tool in fish, normal baseline values will have to be determined much as they are in humans for diagnosing hepatobiliary disease and bone disorders (Tietz, 1986). However, ALP activities appear to be highly variable in fish. Normal range of plasma ALP activities reported for *Clarias Gariepinus* in the present study (table 3). Working on *C. Gariepinus*, our results were in accordance with Shalabi *et al.* (2000) and higher than Mohamed (1981) and lower than Younis, (1993). In the present study, analysis of ALP activity in all groups of *Clarias Gariepinus* administered with the three antibiotics in the three periods studied showed a significant higher values. Casillas and Ames (1986) analyzed the potential use of ALP as possible indicators of liver dysfunction in rainbow trout and English sole respectively. Consistent features of the moribund condition in striped bass identified by Young *et al.* (1994) included elevated ALP level. High ALP levels may reflect the degree of tissue damage seen in specimens of liver,

kidney and intestine from moribund striped bass. Alkaline phosphatase is a membrane enzyme, and the elevated plasma ALP activities measured in *C. gariepinus* in this study corresponded to an inflammatory reaction of the bile ducts. This finding concurs with the results published by Lemaire *et al.* (1991).

In conclusion, although the number of replicates may have been rather low to overcome individual variability, they were not adequate for measuring the possible stress effect of antibiotics. The increases in glucose, urea, creatinine, uric acid and alkaline phosphatase were more clearly defined, and hence it may be that these parameters provide better measures of the stress effect after antibiotic administration. From the results presented here, it can be stated that antibiotics for prophylactic treatment is an under-recognized stress source as, in general, the blood chemistry after antibiotic treatment has undergo alterations.

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Table (1): Mean ± S.D., t-test, ANOVA-test and LSD of different biochemical parameters of *Clarias Gariepius* after the 3rd administration dose (dose/72 hr) of ciprofloxacin, amoxycilline and ampicilline.

Time (day)	Control		Ciprofloxacin		Amoxicillin		Ampicilline		LSD
	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	
	1. Plasma glucose (mg/dL)								
1st day	354.8	24.20	408.1	88.3***Y	601.7	35.2***X	282.7	19.3***	.a
2nd day	328.5	20.00	817.6	6.7***W	346.7	21.3***	253.1	43.4***	.a
3rd day	179.8	9.80	324.2	57.0***	263.2	57.0***	673.7	31.1***X	.a
	C		A		B		B		
	2. Plasma total protein (g/dl)								
1st day	5.48	0.49	6.03	0.03***X	5.92	0.71***	4.46	4.46***X	.a
2nd day	5.64	0.12	5.72	0.28***	5.82	0.49***	5.86	0.65***	.a
3rd day	5.40	0.28	6.71	0.55 X	7.04	0.50*	4.89	0.26***W	.a
	B		A		A		B		
	3. Plasma urea (g/L)								
1st day	0.19	0.01	0.23	0.01***	0.23	0.01***	0.23	0.01***	.b
2nd day	0.21	0.06	0.23	0.01***	0.18	0.04***W	0.24	0.03***	.b
3rd day	0.21	0.03	0.35	0.09***Y	0.31	0.04***Z	0.21	0.01***X	.a
LSD	C		A		B		BC		
	4. Plasma uric acid (g/dl)								
1st day	8.68	0.88	9.31	0.35***	15.91	0.11***X	8.69	0.34 Y	.b
2nd day	7.65	0.30	10.83	0.74***Y	10.47	0.32***Y	10.75	0.12***Y	.b
3rd day	7.85	0.18	17.73	0.34***W	9.66	0.44***	14.68	0.13***X	.a
LSD	C		A		B		B		
	5. Plasma creatinine (g/dl)								
1st day	7.76	1.10	22.68	0.39***W	5.42	0.17***	9.48	0.47***Y	.a
2nd day	4.58	0.73	21.88	0.16***W	6.69	0.05***	10.73	0.28***Y	.b
3rd day	6.67	0.21	9.69	0.10***Y	6.88	0.25	9.38	0.19***Y	.c
LSD	C		A		C		B		

Number of samples in each group = 6 individual. *, **, *** : Significant values compared to control at probability <0.5, <0.01, <0.001 respectively. A, B, C, D : Significant values between groups. a, b, c, d : Significant values between times. W, X, Y, Z: Significant values of interaction between groups and times. N.B.: 1. The 1st letter is highly significant than the second letter ... etc. 2. The same letter means there was non-significant level in comparison.

Table (2): Mean ± S.D., t-test, ANOVA-test and LSD of plasma ALT, AST and ALP of *Clarias Gariepins* after the 3rd administration dose (dose/72 hr) of Ciprofloxacin, amoxycilline and ampicilline.

Time	Control		Ciprofloxacin		Amoxicillin		Ampicilline		LSD
	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	
	1. Alanine aminotransferase(U/L)								
1st day	20.33	0.82	14.50	0.62***	19.66	0.41*	17.00	3.28***	.c
2nd day	21.33	1.03	20.00	0.95	24.66	1.03**Y	19.16	0.41**	.b
3rd day	22.66	1.03	26.00	0.56***X	29.50	3.93***W	20.83	0.41*Z	.a
LSD	B		B		A		B		
	2. Aspartate aminotransferase (U/L)								
1st day	13.17	1.84	15.00	1.09 *Y	13.33	1.03	12.66	0.52	.b
2nd day	17.33	3.62	14.00	1.10 *	19.33	0.83 X	13.66	0.52**	.a
3rd day	14.33	0.52	23.33	0.73***W	19.00	0.44***X	14.67	0.52 X	.a
LSD	B		A		B		B		
	3. Alkaline phosphatase (U/L)								
1st day	9.58	0.77	24.60	0.86***X	16.40	1.27***Y	11.48	0.73***	.a
2nd day	9.64	0.74	25.07	0.05***W	15.16	0.56***Z	16.32	0.73***Y	.a
3rd day	9.61	0.71	23.47	0.52***X	13.94	1.41***	10.39	0.73***	.a
LSD	D		A		B		C		

Number of samples in each group = 6 individual. *, **, *** : Significant values compared to control at probability <0.5, <0.01, <0.001 respectively. A, B, C, D: Significant values between groups. a, b, c, d : Significant values between times. W, X, Y, Z: Significant values of interaction between groups and times. N.B.: 1. The 1st letter is highly significant than the second letter ... etc.2. The same letter means there was non-significant level in comparison.

Table 3: ANOVA and LSD $_{0.5\%}$ values of different biochemical parameters.

	LSD	Glucose		Protein		Urea		Uric acid		Creatinine		ALT		AST		ALP	
		F cal	LSD	F cal	LSD	F cal	LSD	F cal	LSD	F cal	LSD	F cal	LSD	F cal	LSD	F cal	LSD
Group	18	4.93 **	119.	7.85 ***	5.65	7.11 ***	2.29	0.21 NS	3.39	1.58 NS	2.93	9.78 ***	2.81	4.64 ***	2.44	8.77 ***	2.75
Time	56	1.14 NS	103.	12.24 ***	7.49	12.24 ***	6.03	2.42 *	5.45	3.36 *	4.54	2.94 ***	2.43	8.30 ***	2.11	2.98 ***	2.56
interaction	32	9.96 ***	99.5	6.23 ***	6.39	6.23 ***	4.02	3.02 ***	7.42	4.48 ***	6.24	3.71 **	2.18	3.66 **	1.96	3.15 **	2.66