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Disposition Kinetic of Amoxicillin in Healthy and Nephropathic Goats with Immunological and Residual Level in Blood and Tissues

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Abstract

In the present investigation, we evaluated disposition kinetics of amoxicillin in healthy as well as nephropathy black-Bengal goats of either sex through single intravenous (IV) route along with metabolism aspects in all vital organs and urine after repeated therapeutic (TP, 10 mg/kg) and one half of therapeutic dose (HTP, 5 mg/kg) consecutively for 60 days through intramuscular administration (IM), evaluation of blood biochemistry and liver cytochrome p_{450} component. Higher Cl_B, AUC and lower MRT values in nephropathic goats compared to healthy goats indicates rapid distribution and elimination of drug from plasma when amoxicillin was administered to goats with acute renal failure. Continuous administration through intramuscular route up to 60 days revealed biochemical, hematological, histopathological changes in therapeutics as well as below therapeutic dosage level in black-Bengal goats. Present study also revealed immunosuppressive effect during 60 days continuous intramuscular administration and confirmed stimulatory effect on Cytochrome p_{450} enzyme system.

Keywords: Amoxicillin; Pharmacokinetic; Hematological; Biochemical; Immunological studies

Introduction

Amoxicillin, semi synthetic amino penicillin which has activity against penicillin-sensitive gram positive as well as some gram negative bacteria continues to be a useful antimicrobial drug for its low index of toxicity and reliable absorption which continues to make it an attractive agent in the treatment of variety of infections [1].

Pharmacokinetic is concerned with study and characterization of time course of drug absorption, distribution, metabolism and excretion [2]. Disease states alter the pharmacokinetic parameters of many drugs. It has been reported that the kinetic behavior of many drugs were altered following administration by different routes in experimentally induced diseased models of goats and dogs [3-5]. The routine use of conventional dosage regimen in patients suffering from liver and/or renal diseases may result in excessive accumulation of drug in the body and consequently may expose the patient to an excessive risk of serious side effects and toxicity. Considerable interest has arisen in immunotoxicological evaluation of antibiotics and other environmental chemicals in view of their immunosuppressive action. Some of these antibiotics are encountered by the organism over long period of time through food chain. Continuous low level exposure to such antibiotics may increase the susceptibility of the host to various diseases due to immunity breakdown. It has therefore, become important to screen antibiotic at therapeutic dose level with respect to their immunotoxic, biochemical and hematological potential.

So in the present investigation, we evaluated disposition kinetics of amoxicillin after single IV administration in healthy as well as nephropathic black-Bengal goats, recovery from tissues , urine and gastrointestinal tract after repeated therapeutic and one half of therapeutic dose consecutively for 60 days through IM administration, evaluation of blood biochemistry and liver cytochrome p_{450} component.

Materials and Methods

Materials

Amoxicillin was obtained as gift from M/S Alembic Ltd, Mumbai, India. The purity of the compound was 94%.

Animals: Clinically healthy black-Bengal male and female adult goats (1-1.5 year of age) weighing between 12-14 kg was used in this experiment. They were kept individually in custom made stainless steel metabolic cages. The animals were stall fed and water was provided *ad libitum*. Temperature of the animal room was maintained at 22 ± 3°C and provided artificial 12 h light/dark cycle. All experimental protocols were performed in accordance with the University Animal Ethics Committee as stated by WBUAFS.

Induction of kidney damage and pharmacokinetics: Goats of either sex (3 male and 3 female) were administered uranyl nitrate crystals dissolved in distilled water (5%) at 0.75 mg/kg for consecutive 3 days via IV route. On each day blood samples were collected from all the goats to measure the level of BUN and creatinine. On day 4, BUN and creatinine levels achieved twice the normal level which confirmed induction of nephropathy [4]. Once the induction confirmed, amoxicillin was administered intravenously at 10 mg/kg body weight to each nephropathic goats. For healthy kinetics (3 male, 3 female), goats were administered amoxicillin 10 mg/kg body weight IV at a single dose. Blood samples were collected in heparinized test tubes from jugular veins of animals of both the groups at 0 (pre-drug control), 0.08, 0.16, 0.25, 0.33, 0.5, 1.0, 2.0, 4.0, 8.0, 12, 24 and 48 h. Blood samples were extracted and analysed for estimation of amoxicillin level according to established procedure.

Pharmacokinetic parameters were determined from computerized

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curve fitting programme 'PHARMKIT' supplied by the Department of Pharmacology, JIPMER, India.

Metabolism study

Healthy black-Bengal goats were assigned randomly to one of the three groups: the control (C), the therapeutic dose and one half of therapeutic dose group. For the control group, normal saline was used. The TP group received amoxicillin at 10 mg/kg body weight while the HTP group received at 5 mg/kg body weight intramuscularly consecutively for 60 days. Blood samples (6 mL) were collected from jugular vein of each animal of all groups at 0, 14, 28, 42 and 56 day of treatment. Out of 6 mL of collected blood, 1 mL was kept in EDTA vials for haematological study, 1 mL in sodium fluoride for glucose estimation, 1 mL in heparinized tubes for analysis of amoxicillin and rest 3 mL allowed to clot for separation of serum. Serum collected was used for estimation of protein, enzymes and serum immunoglobulin (IgG).

Urine samples (5 mL) were collected from each animal of each group at 0, 14, 28, 42, 56 day of treatment for analysis of urine amoxicillin level. All animals were sacrificed on day 60 and vital organs were collected, frozen immediately until extraction procedure started.

Estimation of biochemical parameters: A small portion of liver and kidney were collected for estimation of reduced glutathione, lipid peroxidation, superoxide dismutase and catalase.

Analysis of drug residue: Portion of lung, liver, kidney, heart, skeletal muscle, adrenal gland, lymphnode, spleen, ovary, uterus, testis and skin were collected from each animal and frozen until analysis of amoxicillin residue.

Histopathology and cytochrome p_{450}: Small portion of liver, kidney and spleen were collected in 10% formol for histopathology and caudate portion of liver was excised and placed in ice cold potassium chloride (1.15%) immediately after slaughter of each animal for estimation of cytochrome p_{450} content.

Instrument and chromatographic condition: Plasma, tissue and urine analysis were performed on a HPLC system (Shimadzu, SPD- M 10 A, Japan) fitted with binary pump (LC-20AT), diode array detector, sampler and data station. A 5 µ Luna Phenomenox (250×4.6 mm) C18 (2) HPLC column was used. The mobile phase consist of methanol and water (1.25% acetic acid) mixed with a ratio of 20:80 (V/V) with a flow rate of 1.5 mL/min and the eluent was monitored with a diode array detector adjusted wave length at 220 nm. Retention time of the drug was 2.50 min. The chromatograms were integrated on a data station.

Recovery experiment: Recovery of amoxicillin from different substrates was carried out *in vitro* to ascertain reliability of the method after fortifying different substrate with technical grade amoxicillin. These were fortified at 1, 5, 25, 50 and 100 ppm of amoxicillin standard and after necessary work up, 20 µL of sample was injected to the HPLC and the area of HPLC peaks against several concentrations was plotted and linearity was found to be maintained. The limit of detection of amoxicillin was 0.01 ppm. The recovery percentage in blood was 94% while in different tissues it was ranged from 81-86% while in urine it was 91%.

Schematic diagrams 1, 2 and 3 presented the extraction procedures of amoxicillin from blood, tissues and urine which was obtained by modified the existing method of Hsu et al. [6].

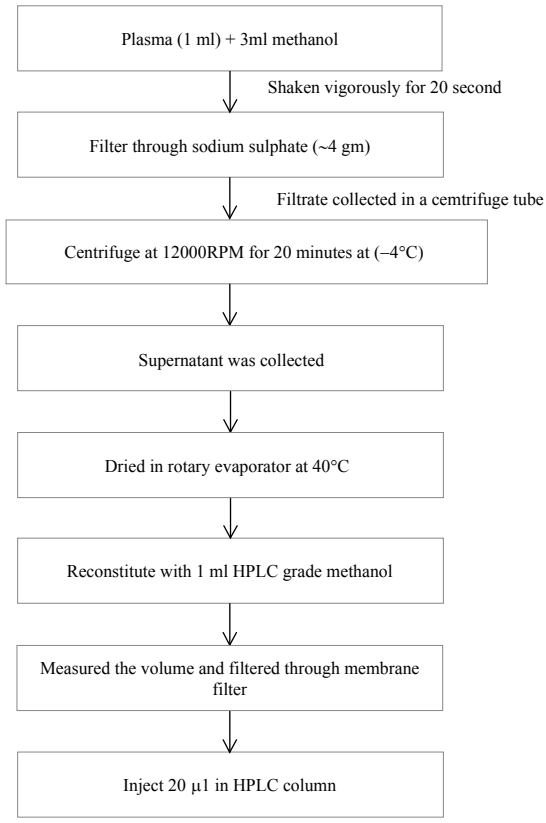
Blood glucose level was estimated as per the method described by Frankel et al. [7], serum protein by Biuret method [8], liver glycogen in liver tissue by Montgomery method [9] while lipid peroxidation was measured by method described by Nair and Turner [10] and reduced glutathione by Griffith [11]. Superoxide dismutase was carried out in accordance with Misera and Fridivich [12] whereas ALT and AST were performed as described by Yatazidis [13]. IgG [14] and estimation of liver microsomal P_{450} [15-17] were done as per standard procedure.

Statistical analysis: All data were expressed as means ± SEM. The differences among groups were considered statistically significant when $p \leq 0.05$ as determined by one way analysis of variance (ANOVA) compared to '0' day values.

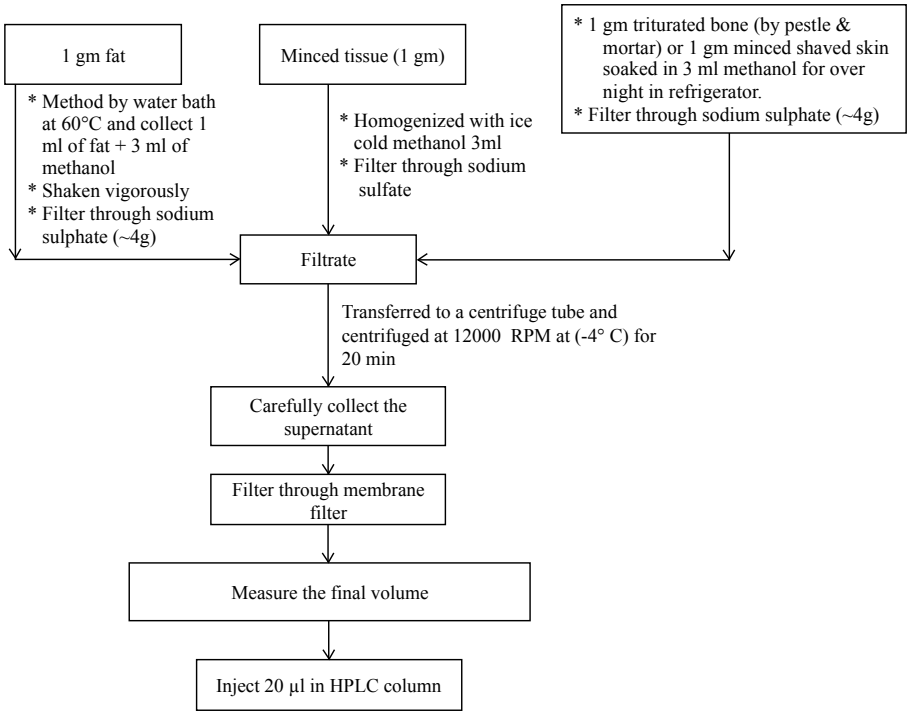
Results

Semi logarithmic plot of mean plasma concentration of amoxicillin against time with computerised best fit line and kinetic parameters in healthy and nephropathic goats following single IV administration at 10 mg/kg is depicted in figure 1 and table 1.

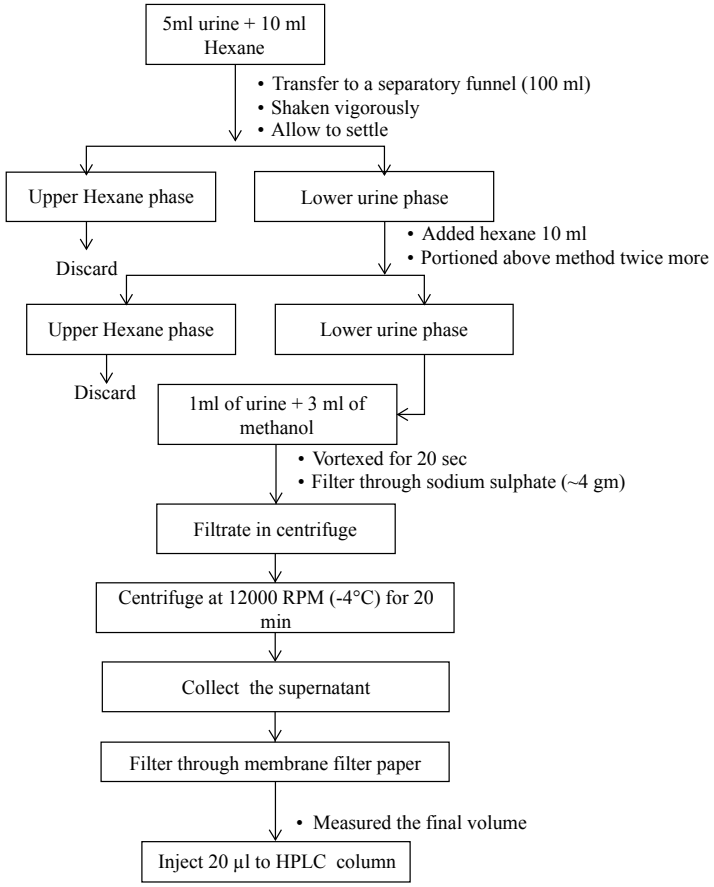
Mean plasma concentration of amoxicillin in TP group at day 14, 28, 42 and 56 were 43.93 ± 6.76, 59.05 ± 8.81, 65.85 ± 8.62, and 70.84 ± 7.20 while in HTP group 41.87 ± 8.48, 53.09 ± 2.91, 55.42 ± 4.72, 51.13 ± 2.64 µg/mL respectively. The mean urine concentration in TP group was 121.89 ± 6.89, 134.45 ± 8.37, 142.73 ± 7.51 and 142.01 ± 8.61 at 14, 28, 42 and 56 day whereas in HTP group values were 74.74 ± 4.07, 97.82 ± 7.46, 101.48 ± 9.35 and 98.49 ± 8.84 µg/mL respectively. Concentration of amoxicillin was remarkably higher in urine compared to plasma in different days of entire collection period for 60 days in both experimental groups.



Schematic diagram 1: Extraction and analysis of amoxicillin from blood.



Schematic diagram 2: Extraction and analysis of amoxicillin from different tissues.



Schematic diagram 3: Extraction and analysis of amoxicillin from urine.

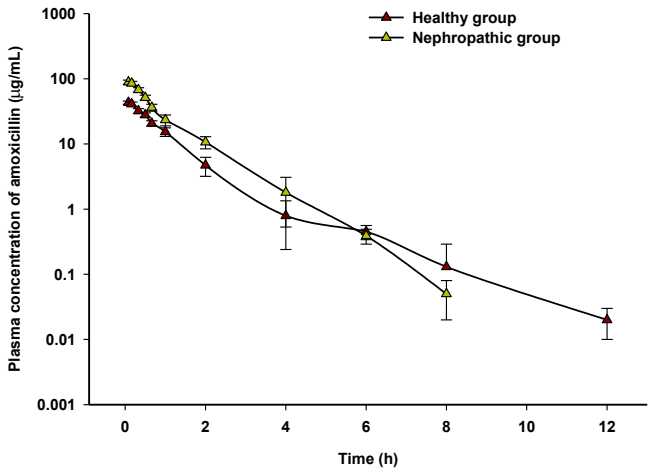


Figure 1: Semilogarithimic plot of mean plasma concentration of amoxicillin against time in healthy and nephropathic goats (n=6, ± SEM) following single dose IV administration at 10 mg/kg.

Parameters	Healthy group	Nephropathy group
α(/hr)	2.31 ± 0.62	3.01* ± 0.78
$t_{1/2\alpha}$ (hr)	0.30 ± 0.10	0.23* ± 0.04
β (/hr)	0.87 ± 0.18	0.58** ± 0.26
$t_{1/2\beta}$ (hr)	0.80 ± 0.21	1.20* ± 0.19
AUC (µg hr /mL)	28.42 ± 4.76	86.04** ± 8.94
MRT (hr)	1.48 ± 0.31	0.97* ± 0.06
K_{21}(/hr)	1.27 ± 0.23	1.38* ± 0.18
K_{12}(/hr)	0.33 ± 0.09	0.95** ± 0.19
K_{el}(/hr)	1.58 ± 0.52	1.27* ± 0.30
Vd_{area}(L/kg)	0.31 ± 0.01	0.19* ± 0.07
Cl_B(ml/h/kg)	22.76 ± 3.41	36.38* ± 7.28

Table 1: Kinetic parameter of amoxicillin in healthy and nephropathic goats after single dose intravenous administration at 10 mg/kg body weight (n=6, ± SEM).**p<0.01, *p<0.05 versus healthy group.

It is apparent from the table 2 that highest concentration of amoxicillin was recovered from kidney (123.93 ± 4.85, 100.31 ± 9.25 µg/gm) followed by liver (109.17 ± 9.35, 97.73 ± 11.65 µg/gm) from both the treated groups. The lowest concentrations were recorded in skin of the TP (6.04 ± 1.21 µg/gm) as well as HTP (7.02 ± 1.46 µg/gm) group.

Mean cytochrome p_{450} contents of control, TP and HTP groups were 0.95 ± 0.007, 1.22 ± 0.09 and 1.31 ± 0.18 nmol/mg of microsomal protein respectively. Significant inductions were noticed in both the experimental groups compared to control group.

The haemoglobin level was decreased significantly in TP group (Table 3a) on day 56 (7.70 ± 0.72 gm/dL) compared to day 0 (8.69 ± 0.60 gm/dL). The total erythrocyte count in animals of TP and HTP groups on day 42 (8.58 ± 0.38, 8.74 ± 0.47×10^{12}/L) and onwards decreased significantly compared to value of day 0 (Table 3b), whereas in the values of total leucocyte count was found to be decreased significantly from day 42 (8.47 ± 0.61×10^9/L) onwards in TP and day 56 in HTP (8.26 ± 0.28×10^9/L) group (Table 3c).

The blood glucose values (Figure 2A) in animals of TP and HTP group on day 56 (52.93 ± 1.29 and 50.99 ± 1.08 m mole/L) was significantly decreased compared to day 0 (59.67 ± 0.64, 58.85 ± 1.16 m mole/L). The serum ALT and AST (Figures 2B and 2C) activities in TP group was found to be increased significantly from day 42 (30.48 ± 2.09, 128.42 ± 4.7 µg pyruvic acid/mL/h) and onwards while in HTP group significant increase was recorded on day 56 (32.09 ± 2.5, 127.13 ± 5.0 µg pyruvic acid/mL/h). The serum protein level of TP group was found to be differ significantly only on day 56 (52.91 ± 3.8 gm/L) of experiment (Figure 2D) while serum immunoglobulin (IgG) levels (Figure 3) found to be differ significantly (P<0.05) from day 42 (8.57 ± 1.0, 8.28 ± 0.76 mg/mL) and onwards of experiment in both TP and HTP groups. No significant differences were found in tissue biochemistry (result not presented).

Discussion

Present investigation reported higher V_{darea} (0.31 ± 0.01 L/kg) and lower Cl_B (22.76 ± 3.41 mL/h/kg) in healthy goats (Table 1) which were consonance with the finding of Frankel et al. [7] and Errecalde et al.

Tissues/ Groups	TP	HTP
Kidney	123.96 ± 4.85	100.31 ± 9.25
Liver	109.17 ± 9.35	97.73 ± 11.65
Lung	44.48 ± 5.66	22.34 ± 3.21
Muscle	61.98 ± 6.03	45.86 ± 3.56
Skin	6.04 ± 1.21	7.02 ± 1.46
Brain	36.94 ± 9.69	21.69 ± 1.82
Spleen	77.72 ± 9.46	53.28 ± 12.11
Heart	69.16 ± 9.05	81.53 ± 11.98
Bile	83.89 ± 9.29	71.72 ± 7.98
Bone	29.14 ± 3.15	24.16 ± 2.29
Uterus	105.94 ± 14.93	49.29 ± 7.88
Testes	63.47 ± 11.72	51.69 ± 7.75
Ovary	48.91 ± 7.77	35.92 ± 8.50
Adrenal Gland	88.27 ± 11.93	56.83 ± 4.71
Large Intestine	104.57 ± 16.42	94.50 ± 7.99
Small Intestine	93.48 ± 10.42	94.73 ± 9.41

Table 2: Mean tissue concentration (µg/gm) of amoxicillin at the end of continuous administration for 60 days in two dose level.

Group/Day	0	14	28	42	56
Control	8.48^a ± 0.67	8.42^a ± 0.69	8.32^a ± 0.71	8.51^a ± 0.36	8.37^a ± 0.36
TP	8.69^a ± 0.60	8.36^{ab} ± 0.29	8.05^{ab} ± 0.76	8.00^{ab} ± 0.82	7.70^b ± 0.72
HTP	8.25^a ± 0.42	7.92^{ab} ± 0.28	7.86^{ab} ± 0.41	7.56^{ab} ± 0.37	7.61^b ± 0.41

P<0.05, values with at least one similar superscript do not vary significantly from day 0

Table 3a: Effect of amoxicillin on Haemoglobin (gm/dL) level on goats during long term administration for 60 days in two dose level.

Group/Day	0	14	28	42	56
Control	10.14^a ± 0.65	10.56^a ± 0.41	10.17^a ± 0.83	10.71^a ± 0.79	10.32^a ± 0.49
TP	9.91^a ± 0.49	9.63^{ab} ± 0.45	8.87^{ab} ± 0.33	8.58^b ± 0.38	8.74^b ± 0.21
HTP	10.39^a ± 0.17	9.83^a ± 0.27	9.21^{ab} ± 0.47	8.74^b ± 0.47	8.26^b ± 0.28

P<0.05, values with at least one similar superscript do not vary significantly from day 0

Table 3b: Effect of amoxicillin on Total Erythrocyte Count (x 10^{12}/L) on goats during long term administration for 60 days in two dose level.

Group/Day	0	14	28	42	56
Control	10.52^a ± 0.34	10.76^a ± 0.42	10.61^a ± 0.30	10.79^a ± 0.59	10.81^a ± 0.52
TP	10.10^a ± 0.78	9.99^{ab} ± 0.36	9.06^{ab} ± 0.32	8.47^{bc} ± 0.61	7.57^c ± 0.48
HTP	10.92^a ± 0.54	10.34^{ab} ± 0.77	9.82^{ab} ± 0.67	9.52^{ab} ± 0.40	8.60^b ± 0.21

P<0.05, values with at least one similar superscript do not vary significantly from day 0

Table 3c: Effect of amoxicillin on Total Leucocytes Count (x 10^9/L) on goats during long term administration for 60 days in two dose level.

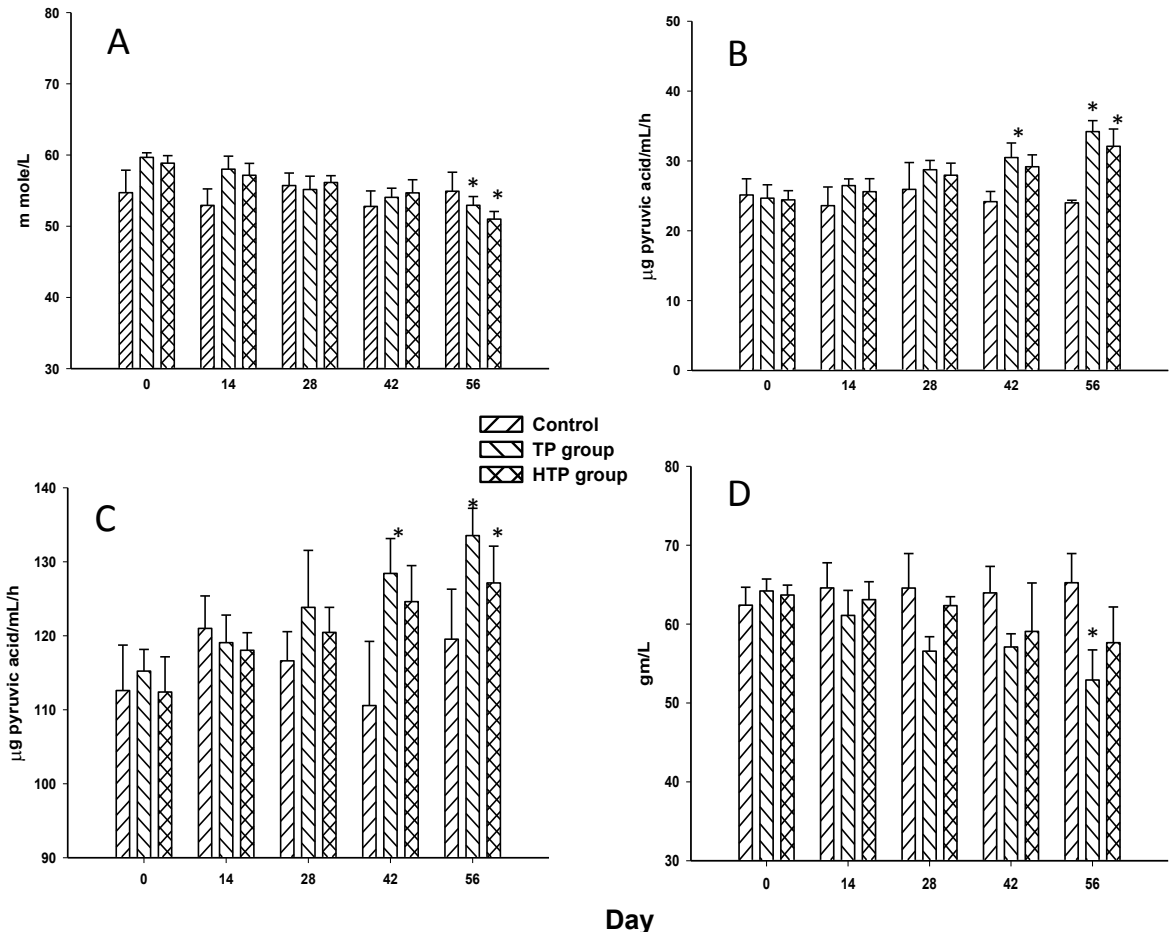


Figure 2: Effect of amoxicillin following consecutive once daily IM administration up to 60 days. Panel A illustrates blood glucose (m mole/L) level; panel B illustrates alanine transaminase level (µg pyruvic acid/mL/h), panel C illustrates aspartate transaminase level (µg pyruvic acid/mL/h) and panel D describes serum protein level (g/mL). $P \leq 0.05$ compared to day 0.

[18] after single IV administration of amoxicillin in healthy goats and normal horse. Shaktidevan et al. [19] reported significantly higher AUC and lower β and Cl_B in acute renal failure goats compared to healthy goats supported present findings. Higher Cl_B values in nephropathic goats compared to healthy goats indicates rapid elimination of drug in kidney damaged goats. Major excretory pathways of most of the penicillin are directed through kidney particularly via glomerular filtration and active tubular secretion [20]. Again rapid distribution and elimination of drug from plasma were found when amoxicillin was administered to goats with acute renal failure. A low value of K_{12} was obtained in both healthy and nephropathic goats. These values are remarkably lower than obtained for K_{21} values suggested that micro constant associated with amoxicillin flow towards central compartment is high. In most cases, the drug concentration in plasma samples at different time intervals in nephropathic goats were significantly higher than those in healthy goats up to 6 h (Figure 1).

The plasma and urine concentration of amoxicillin in TP group was higher compared to HTP group during almost entire collection period suggesting dose dependent increase of drug concentration in blood and urine. Excretion of amoxicillin was mainly via kidney resulting high concentration in urine compared to blood. Similar findings were observed by Satoskar and Bhandarkar [21] in urine following administration of β-lactam antibiotic.

It is evident from the result that amoxicillin is inducer of hepatic microsomal mixed function oxidase system. High levels of MFO are present in liver. Amoxicillin might cause hydrolysis of MFO system which may lead to induction of cytochrome p_{450} content [22] or may lead higher intrinsic microsomal resulting in increase the level of Cytochrome p_{450} level. Huwyler et al. [23] also reported stimulation of microsomal enzymes after administration of β-lactam antibiotic for prolong periods.

The lower values of Hb, TEC, TLC indicates anemic trend with consecutive daily IM administration of amoxicillin at two dose level in the experimental animals which is possibly due to depression of bone marrow and or suppression of haemopoietic tissues. Ito et al. [24] also found a reduce level of Hb after sub-cutaneous injection of beta lactam antibiotic for consecutive 26 weeks at different dose levels in rats.

It is transpired from the Figure 2A that amoxicillin caused hypoglycaemia on day 56. Similar results were obtained by Ito et al. [24] after consecutive administration of β-lactam antibiotic for 26 weeks. The liver is a key to glucose homeostasis: any disruption of its metabolism, structural integrity or intracellular dynamics may alter liver's ability to maintain normal glucose homoeostasis. When such disruption affects hepatic glucose output, hypoglycemia may eventuate [25]. In the present investigation, histopathology of liver reveals degenerative changes (Figures 4A and 4B) which might be responsible

for hypoglycemia. The decreased trend of protein (Figure 2D) level might be due to the fact that hypoglycaemic animals tried to make up the glucose level by catabolising protein.

The increased level of ALT (Figure 2B) in blood may be due to pathological changes like proliferation of Glissons capsule (Figures 4A and 4B) in liver tissue. Due to prolong administration of amoxicillin, permeability of hepatocyte cell may be increased and resulting to escape of this enzyme into blood leading to higher level of enzyme in the present study. Increased serum ALT and AST (Figures 2B and 2C) activity are particularly marked in infective hepatitis and in liver damage due to drugs or chemicals [8]. Chronic administration of amoxicillin may cause increased permeability of cell membrane resulting in increased activity of serum. Decreased IgG (Figure 3) levels were suggested that amoxicillin has the immunosuppressive effect during long term administration.

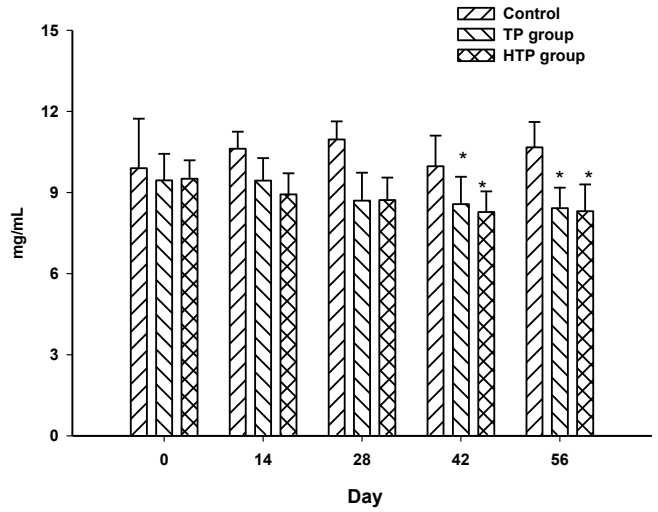


Figure 3: Effect of amoxicillin following consecutive once daily IM administration up to 60 days on serum immunoglobulin level (mg / mL). $P \leq 0.05$ compared to day 0.

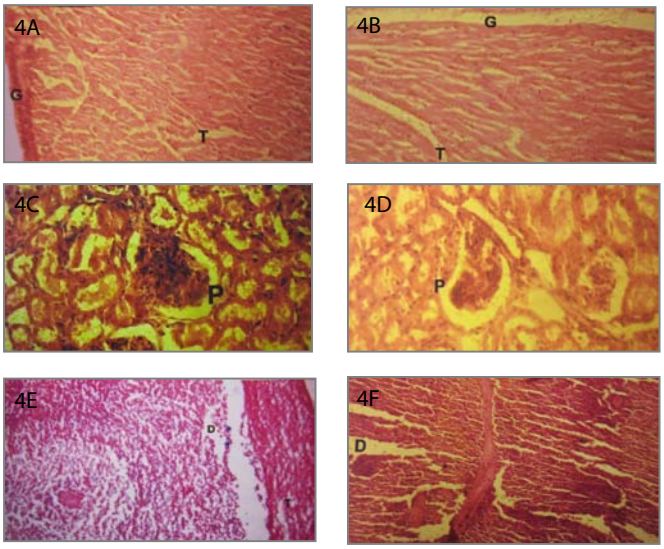


Figure 4: Section of liver of TP group (4A) and HTP group (4B) showing cirrhotic changes with proliferation of Glisons capsule and connective tissue (G,T). Section of kidney of TP group (4C) and HTP (4D) group showing connective tissue proliferation (P). Spleen section of TP group (4E) and HTP group (4F) showing marked depletion of lymphocyte (D) and thickening of capsule (T).

Histopathology of liver (Figure 4) revealed that both TP and HTP dosages lead to fatty changes and fatty vacuoles pressed the nucleus of the hepatocytes whereas kidney of the groups revealed marked thickening of renal capsules which were absent in control group (not presented) suggested long term administration has harmful effect on these organs.

From the finding of the present study, it can be concluded that the usage of amoxicillin should not be recommended in nephropathic condition as well as for consecutive long term administration and further evaluation is necessary to clarify the potential application of amoxicillin in mentioned circumstances.

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