Bioavailability and Disposition Kinetics of Amoxicillin in Normal and Febrile Rabbits

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ABSTRACT

Objective: The study was planed to see bioavailability and disposition kinetics of amoxicillin in adult rabbits (irrespective of sex) under healthy and induced febrile conditions.
Design: Comparative
Place and duration of study: Study was conducted at the department of pharmacology, University of Veterinary and Animal Sciences, Lahore from January 2003 to July 2003.
Methodology: Initially all rabbits were weighed and their packed cell volume (PCV) and other biochemical parameters were observed in normal conditions. Bioavailability and disposition kinetics of amoxicillin (10mg \ Kg body weight) were studied in all normal rabbits following oral and intravenous route of drug administration. A wash out period of 10 days was given in between the two trials. Fever was produced by injecting the E. Coli suspension (prepared in sterilized water) in the marginal ear vein of rabbits at a dosage rate of 0.01 ml per Kg body weight. Blood samples were collected from the rabbits suffering from fever to determine packed cell volume and biochemical parameters. Treated rabbits were administered amoxicillin orally and intravenously (10 mg /Kg body weight). Rectal temperature and behavioral changes were recorded and samples were drawn at prescribed time intervals. Amoxicillin was assessed in plasma by using microbiological assay method. Plasma concentration was analyzed using non compartmental method.
Results: A non significant difference was found in biochemical parameters and body weight was found except total lipids. There was significant decrease in ($P < 0.01$) packed cell volume and plasma concentration of amoxicillin of the febrile rabbits. A statistically significant ($P > 0.05$) increase in the total lipids was observed in febrile rabbits. The plasma drug concentration of orally administered amoxicillin was significantly lower ($p< 0.01$) in febrile rabbits. Where as no difference was observed in the intravenous administration of amoxicillin in normal and febrile rabbits. Clearance of the drug was significantly lower in the febrile group. Endotoxin induced fever in rabbits on the biochemical and pharmacokinetic parameters of amoxicillin revealed that in rabbits during Endotoxin induced fever, packed cell volume, total lipids and plasma concentration of amoxicillin decreases significantly ($p<0.01$). The plasma drug concentration of orally administered amoxicillin was significantly lower ($P<0.01$) in febrile rabbits. The lower plasma drug levels resulted to a smaller area under curve, a larger volume of distribution and a higher clearance value in febrile rabbits. Where as not much difference was observed in the intravenous administration of amoxicillin in normal and febrile rabbits. Clearance of the drug was significantly lower in febrile group placation
Conclusion: This study in rabbits indicates the need of modification of dosage regimen under experimentally induced febrile state and warrant clinical evaluation /application.

Key Word: Amoxicillin, Febrile

INTRODUCTION

Amoxicillin is a broad spectrum antibiotic. It is active against gram negative and gram positive bacteria including Streptococcus SPP, Haemophilus influenza, E. coli, Proteus mirabilis, Salmonella SPP, and Nisseria SPP. It is a drug of choice in Gonorrhoea , typhoid , paratyphoid and meningitis. Amoxicillin continues to be a useful antimicrobial drug and its low index of toxicity, freedom from sensitization and reliable absorption continues to make it an attractive agent in the treatment of variety of infections[1].

Several diseased conditions lead to biochemical alteration in the milieu interior and affect bioavailability and disposition kinetics of drugs. For example biodisposition of sulfadimidine has been shown to be different in normal and febrile dogs[2]. Fever is an important symptom in several infectious diseases in man and animals. When the fever is induced by Endotoxin, it is likely that kinetics of drugs

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during hypothermic process will be changed[3]. It is important therefore to determine whether fever itself consistently changes the way healthy individuals handle amoxicillin.

METHODOLOGY

A total no of 12 healthy, adult rabbits of either sex were obtained from Department of live stock Management, University of Veterinary and Animal Sciences, Lahore, A ten days period was allowed to condition the animal for handling and adaptation. The animals were routinely examined by veterinarian. The average body weight of the animals was 1.51±0.34Kg ranging between 1.2 – 1.89 Kg. The animals were used for normal study and then used for experimental conditions.

Fever was produced by injecting the E. Coli suspension in marginal ear vein of rabbits at a dosage rate of 0.01 ml per Kg body weight. Rectal temperature was recorded before and at 5, 15, 45, 120, 240, 360 and 480 minutes time interval after the drug administration during experiment. Initially all the rabbits were weighed and their blood pH, blood glucose, total plasma protein, total plasma lipids, albumin, globulin ratio was determined. Bioavailability and disposition kinetics of amoxicillin were studied in all normal rabbits following intravenous injection (10 or 50 mg /Kg body weight) and oral route of drug administration. Blood samples (2ml) were drawn from the jugular vein at 5, 10, 15, 30, 45, 60, 120, 240, 360 and 480 minutes.

A wash out period of 10 days was given after completion of bioavailability and disposition kinetics trial in normal animals. The experimental diseased condition was induced in all rabbits. The blood samples were collected from the rabbits suffering from fever to determine their packed cell volume and pH, blood glucose, total lipids, total proteins albumin and globulin after the administration of amoxicillin through oral and intravenous route separately. For determining the bioavailability and disposition kinetics of amoxicillin in normal and treated rabbits the drug was administered as a single dose 10mg /Kg body weight. The blood samples were collected at 15, 30, 45, 60, 120, 240, 360 and 480 minutes. After the drug administration, Blood was collected in heparinzed glass centrifuge tubes and plasma was separated and used for analysis. Amoxicillin concentration in plasma was measured by a microbiological assay by using sarcina lutea (ATCL 9341) as test organism[4,5].. Plasma levels of amoxicillin in normal and febrile rabbits were used to analyse their individual kinetic parameters using the non- compartmental method of analysis and the results were compared by student T test[6].

RESULTS

The influence of febrile state on various biochemical parameters of blood and biodisposition kinetic parameters of amoxicillin was investigated in rabbits. The mean results showing body weight, packed cell volume, biochemical parameters, plasma concentration and biodisposition in normal and experimentally diseased rabbits are as follows.

The normal body temperature in normal rabbits ranged between 101 F ^{0} – 102.5 F ^{0}.

The rectal temperature after the administration of a suspension of E. Coli for the production of fever reached to maximum of 104^{0}F. The mean ±S D rectal temperature of the febrile rabbits was 103.5°F.

The biochemical parameters, body weight and pack cell volume we determined in the normal rabbit. These animals were then treated with suspension of E. Coli to produce the febrile condition during which the body weight, pack cell volume and biochemical parameters were measured again. All the results have been compared statistically (Table 1).

A non significant difference was found in body weight, blood pH, total protein, albumin, globulins, and albumin / globulin ratio in normal and febrile groups. There was significant ($P<0.01$) decrease in the pack cell volume of febrile rabbits. A fall of 20% was observed in the packed cell of the febrile rabbits. A statistically significant ($p<0.05$) increase in total lipids of the febrile rabbits was also observed. Following oral administration of amoxicillin suspension (10 mg/kg) in normal and fever induced rabbits, plasma concentration was measured. All these concentration has been compared statistically in table No 2 and found to be significantly (p,0.05) and (p,0.01) lower in the febrile rabbits at all times except for the last sampling time that means 480 minutes after the drug administration. The peak plasma concentration of 6.72µg / ml in the normal rabbits was attained after 30 minutes, while maximum plasma concentration of 3.04 µg / ml was reached after 60 minutes in the febrile rabbits. Plasma concentration of amoxicillin following intra venous administration (10mg / kg body weight) in normal and febrile rabbits was observed. The statistical comparison was performed individually at each time interval (Table 3). It was observed that the plasma concentration of febrile rabbits were significantly ($p < 0.01$) and ($p < 0.05$) higher from the plasma concentration of the normal rabbits at 60, 120 and 240 minutes after drug administration. The concentration at remaining time intervals of sampling that means 5, 15, 30, 45, 360 and 480 minutes were found to be non significantly different. The plasma concentration ranged between 8.02 ± 0.55 and 3.12 ± 0.77 µg / ml in febrile rabbits.

Table 1: Mean = S.D. (n =10) values for the body weight, packed cell volume and biochemical parameters of blood in normal and febrile rabbits.

Parameters	Normal	Febrile	Group
Body weight	Kg	1.51+ 0.34	1.57+0.32
Packed cell vol.	%	36.00+3.52	30.30+3.63**
Blood pH	7.25+0.05	7.28+0.04	
Blood glucose	mg/dl	110.00+19.0	109.00+12.0
Total lipids	mg/dl	524.00 + 61.0	585.00+71.0
Total proteins	g/dl	7.80+0.51	7.65+0.82
Albumins	g/dl	3.95+0.35	3.95+0.40
Globulins	g/dl	3.85+0.11	3.70+0.42
A/G ratio	1.02+0.34	1.07+0.95	

* = Significant difference (P < 0.05), ** = Highly significant difference (P < 0.01)

Table 2: Mean + S.D (n = 10) plasma concentration (ug /ml) of amoxicillin following oral administration of 10 mg / kg dose in normal and febrile rabbits.

Time (minutes)	**Normal**	**Febrile**
5	3.93+0.34	2.87+0.07*
10	5.09+0.27	2.90+0.04**
15	6.21+0.26	2.93+0.04**
30	6.72+0.29	2.94+0.04**
45	6.38+0.28	3.02+0.08**
60	5.67+0.27	3.04+0.12**
120	4.86+0.26	2.82+0.10**
240	3.89+0.28	2.71+0.10**
360	3.06+0.42	2.66+0.05**
480	2.70+0.30	2.4+0.19

* = Significant difference (P < 0.05) ** = Highly significant difference (P < 0.01)

Table 3: Mean+S.D (n = 10) plasma concentration (ug / ml) of amoxicillin following intravenous administration of 10 mg / kg dose in normal and febrile rabbits. Groups

Time (minutes)	Normal	Febrile
5	8.02+0.39	8.10+0.55
15	7.33+1.22	7.60+0.12
30	6.60+1.19	6.83+0.40
45	5.88+0.97	6.37+0.38
60	5.13+0.68	5.88+0.41**
120	4.38+0.50	4.92+0.54*
240	3.66+0.38	4.09+0.49*
360	2.99+0.27	3.26+0.56
480	2.58+0.37	3.12+0.77

* = Significant difference (P < 0.05) ** = Highly significant difference (P < 0.01)

Table: 4. Mean = S.D. (n =10) values for bioavailability and disposition kinetics of amoxicillin following oral administration of 10 mg/kg dose in normal and febrile rabbits.

Parameters	**Units**	**Normal**	**Febrile**
AUC	µg/h/ml/kg	26.30+7.20	16.60+5.30**
AUMC	µg /h2/ml/kg	69.30+33.4	54.0+26.0
K-termina	/hr	0.41+0.09	0.34+0.10
T ½	hrs	1.75+0.40	2.12+0.50
MRT	hrs	2.52+0.54	3.06+0.70
C1	ml/hr/kg	97.80+12.00	193.0+42.0**
Vd	1/kg	0.41+0.11	0.69+0.33*
T (max)	minutes	30.00	30.00
C (max)	µg /ml	6.72+0.30	2.94+0.40
F	%	0.90	0.57

* = Significant difference (P < 0.05), ** = Highly significant difference (P < 0.01)

Table 5. Mean = S.D. (n =10) values for disposition kinetics of amoxicillin following Intravenous administration of 10 mg / kg dose in normal and febrile rabbits.

Parameters	Units	Normal	Febrile
AUC	µg / h /ml /kg	29.02+4.00	29.10+6.10
AUMC	µg /h2 /ml /kg	93.00+22.00	84.00+33.60
K-termina/hr	/hr	0.32+0.05	0.38+0.08
T ½	hrs	2.20+0.32	1.93+0.97
MRT	hrs	3.17+0.46	2.78+0.56
C1	ml/hr/kg	110.0+14.70	96.0+6.33*
Vd	1/kg	0.35+0.05	0.36+0.08

* = Significant difference (P < 0.05)

DISCUSSION

Fever means a body temperature above the usual range of the normal, may be caused by abnormalities in the brain it self or by toxic substances that affect the temperature regulating centers. Some causes of fever include bacterial diseases, brain tumors and environmental condition that may terminate in heat shock[7]. Fever is accompanied with dullness, headache, shivering and laziness. There are circulation changes, altered heart rate, cardiac out put and increase in blood flow through liver and kidney and a cutaneous vasoconstriction.[7] Disturbances of gastric motility and secretion of gastric juices has been observed during fever in mono gastric as well as poly gastric animals[8,9]. Pyrogen induced fever has long been used as the experimental tool to study the pharmacokinetics and bioavailability of different compounds in various animals, such as influence of fever on the pharmacokinetics of amoxicillin in febrile beagle dogs[10]. Erythromycin in rabbits[11] and sulfadimidine in dogs[12,13,14], Glucose in dogs[15], Antipyrine in humans[16] quinine in human[17] and salicylamide in human[18].

Body weight of the normal and the E. Coli treated febrile rabbits did not reveal any difference as was observed while studying the bioavailability and disposition kinetics of erythromycin in normal and febrile rabbits. A highly significant (p<0.01) decrease in packed cell volume of the febrile rabbits may be due to increased cell destruction in the presence of Endotoxin. Total lipids were significantly (p<0.05) higher in the febrile group which may possibly be attributed to the catecholamine induced increased lipolysis or fat metabolism. Blood pH , blood glucose, total protein, plasma albumins and globulins were similar in normal and febrile rabbits. Ahmad [11] also reported unaltered values of blood pH , blood glucose, plasma albumin and globulins in his studies made with Erythrosine in normal and febrile rabbits. Plasma concentration after oral administration of amoxicillin suspension revealed significant (P<0.05 & P <0.01) difference in normal and Endotoxin induced febrile rabbit, plasma concentrations were lower in febrile group throughout the sampling period showing a very poor absorption from the stomach which is contrary to common belief, that the stomach is not important site of drug absorption.[19, 20] However, the rate of gastric emptying can markedly influence the rate at which drugs are absorbed and any disease or drug which influences this process may also influence the rate of absorption (and in turn the onset of pharmacotherapeutic action) of an orally administered agent[21,22]. It has been demonstrated that E. Coli can cause inhibition of gastric secretions[23,24,25] and of gastric emptying rate [26] in mono gastric animals. According to the pH partition hypothesis the absorption of weakly acidic drugs, like the sulphonamides and aspirin should be slowed in chlorhydric patients, but in fact in one study aspirin was absorbed significantly faster and plasma salicylate concentrations were higher than in controls[20]. On the other head the absorption rate of amoxicillin was delayed in febrile dairy calves (E Coli 0.01 mg per kg body weight intravenously) after oral drug administration[2] as has been observed in this study. E. Coli is a strong inhibitor of reticuloruminal contraction in sheep and goat. [9] Consistent lower plasma concentration in febrile group also been describe by Leenan and Van Miert[25]. According to them, E. coli can cause inhibition of gastric secretion and emptying, but due to lack of motility of the drug molecules which are not coming in full contact with the absorption surface, so the drugs which are administered orally can not reach to the systemic circulation. After intravenous administration the plasma concentration of amoxicillin in normal rabbits were compared with the febrile rabbits. There was statistically significant difference (P < 0.05 & 0.01) at 60, 120 and 240 minutes after drug administration, where all the plasma concentration in the Endotoxin induced febrile group were all the times higher that the normal group. These differences in the plasma concentration can be explained on the fact that the percentage of amoxicillin binding to proteins depends upon the concentration and percentage of protein binding drugs which in turn depends upon the concentration of alpha 1 acid glycoprotein's in

plasma. In physiological concentration of human serum albumin and alpha 1 glycoprotein bind 8.7 % and 54.4% of the drug respectively. As alpha1 acid glycoprotein increased resulting from an inflammation in infectious states[27]. Though the plasma protein binding of amoxicillin was not measured but it can be guessed by the increased plasma concentration in the febrile group after intravenous administration. Amoxicillin binding increased in case of an increased body temperature resulting it into high plasma concentration then the normal rabbits. Bioavailability and disposition kinetic parameters of amoxicillin were analyzed and the results are shown in table 4 & 5.After oral administration total area under the plasma concentration time curve show a significantly ($P<0.01$) lower value in the febrile group due to the lower plasma concentration of amoxicillin. As the eliminate (kel) ion rate constant was not statistically different in the normal and febrile group, so the half life and mean residence time were not different in both the groups. Total body clearance which is the amount of drug being cleared from the body in a unit time was significantly ($P<0.01$) higher in the Endotoxin induced febrile group. The results are quite in agreement with the results of Lashev et al[28]. Who also observed an increased clearance through the oral route of administration?

Volume of distribution (Vd) showed a significant ($P< 0.05$) rise in the Endotoxin induced febrile group. A higher value of Vd very well explain the tissue penetration of some antimicrobial agents, reflected by an increased magnitude of the volume of distribution in Endotoxin induced fever where an acute type of infection is present. This situation was found to exist for penicillin G, when the disposition kinetics of the drugs were compared in normal beagles during acute stage, based on febrile response of an induced streptococcal infection by Baggot[29]. A greater volume of distribution of warfar and trimethoprim in rabbit was also observed during Endotoxin induced fever[30],[13].

The time taken by the drug to reach its maximum concentration (T max) in the body was the same in the normal and the febrile rabbit but there was a significant difference in the maximum plasma drug concentration (C max) in the normal and febrile rabbit. It was significantly higher ($P < 0.01$) in the normal rabbits. In a previous study made by Van Miert[31] Leek and Van Miert[9] plasma concentration was all the time higher in the normal sheep and goat as compared to the febrile animals. Fraction of total dose of amoxicillin available in normal rabbits was 90% whereas in the febrile rabbits it was 57% of the total dose. These values were significantly different from each other confirming the previous recommendations made by Riffat et al[32], Nawaz[33], Nawaz & Shah[34] of a different dosage regimen in diseased animals. After intravenous administration of amoxicillin the plasma concentration before and after Endotoxin induced fever did not differ. Therefore, the disposition and kinetic parameters also did not show any difference between normal and febrile rabbits which is similar to the observations made in febrile rabbit[35] in goats and sheep. With high body temperature due to Endotoxin [3] in dogs with E. Coli induced fever[2] the value of total body clearance increased significantly ($P < 0.05$) in febrile rabbits. Total body clearance increased significantly ($P<0.05$) in febrile rabbits. Total body clearance is a direct measure of hepatic elimination regardless of the number of compartments a drug becomes distributed into the body. This value was quite in consideration with another study made in febrile rabbits[11]. These studies in rabbits indicate the need for modification of dosage regimen under experimentally induced fever.

CONCLUSION

This study in rabbits indicates the need of modification of dosage regimen under experimentally induced febrile state and warrant clinical evaluation /application.

REFERENCES

1. Henry. F. Chambers. Beta- Lactam & other cell wall & Memberane- Active Antibiotics.In: Bertram G. Katzung, eds. Basic and Clinical Pharmacology, 8th Ed. Connecticut: Appleton and Lange 2001; 757 - 761.
2. Riffat, S., Nawaz, M. and Rehman, Z. Pharmacokinetics of sulphadimidine in normal and febrile dogs. J. Vet. Pharmacol. Therap. 5: 131- 135, 1982.
3. Van Gogh, H and Van Miert, A .S. J. P. A. M. The absorption of sulphonamides from gastrointestinal tract during pyrogen induced fever in kids and goats. Zbl. Vet. Med. A., 24: 503 – 410, 1977.
4. Kanazawa, Y. T., Kuramata, K. And Matsomoto, K.A study on the disc sensitivity test for amoxicillin Jpn. J.Antibiot. 37 (9) : 1661 – 1668, 1984.
5. Semenitz, E., Casey, P. A., Pfaller, W., Gstraunthaler, G. Microcalorimeteric, turbidimetric, phase contract, microscopic and electron Microscopic investigation of the action of amoxicillin. Chemotherapy. 29(3): 192 – 207, 1983.
6. Gibaldi, M. Biopharmaceutics and Clinical Pharmacokinetics, 3rd Edition, Lea & Febriger, Philadelphia, 1984.
7. Guyton, A. C. A Testbook of Medical Physiology. 4th Edition, W. B. Saunders & Co., Philadelphia, pp. 915 – 928, 1971.
8. Ballard, B.E. Pharmacokinetics and temperature. J. Pharm. Sci. 63: 1345 – 1358, 1974.
9. Leek, B. F. and Van Miert, A. S. J. P. A. M. An analysis of the pyrogen induced Inhibition of gastric motility in

sheep. J. Physiol. 215: 28 – 29, 1971.
10. J. F. Marier, F. Beaudry M.P. Ducharme, D. Fortin, J. P. Moreau, R. Masse & P. Vachon. A pharmacokinetics study of Amoxicillin in febrile beagle dogs following repeated administrations of Endotoxin. Journal of Veternary Pharmacology and Therapeutics. 24: 379 - 383, 2002.
11. Ahmed, M. Bioavailability and disposition kinetics of erythromycin in normal, febrile, metabolically altered and water deprived rabbits.Ph. D. Thesis, Univ. of Punjab, Lahore, 1990.
12. Ladefoged, O. pharmacokinetics of trimethprim (TMP) in normal and Febrile rabbits. Acta. Pharmacol. Et. Toxicol. 41: 507 – 514, 1977.
13. Ladefoged, O. The effect of E. coil Endotoxin on the G. I. absorption of Antipyrene in rabbits. XIII. Nord. Vet. Congr. Abo. P. 363, 1978a.
14. Ladefoged, O. Endotoxin induced changes in pharmacokinetics of warfarin in rabbits. Acta. Vet. Scand. 479 – 486, 1978b.
15. Wolf, R. R., Elahi, D. and Spitzer , J.J Glucose kinetics in dogs following a lethal dose of Endotoxin. Metabolism. 26: 847 – 850, 1977.
16. Elin, R. J., Vessel, E.S. and Wolf, S. M. Effect of ethiochlanalone induced fever on plasmaantipyrene half life and metabolic clearance. Clin. Pharmacol.
17. Therap. 17: 447 – 457, 1975.
18. Trenholm, G. M., R. J., Rieckman, K. H., Frischer, H. and Carson, P. E. Quinine disposition during malaria and during induced fever. Clin. Pharmacol. Therap. 19: 459 – 467, 1976.
19. Song, C. S., Gelb, N. A. and Wolf, S. M. The influence of pyrogen induced fever on the salicylamide metabolism in man. J. Clin. Invest. 51: 2959 – 2966,
20. Heading, R. C., Nimmo, J., Prescot, L. F. and Tothill, P. The dependence of paracetamol absorption on the rate of gastric emptying. Br. J. Pharmacol, 47: 414 – 421, 1973.
21. Pottage, A. W., Nimmo, W. S. And Prescott, L. F. The absorption of aspirin and paracetamol in patients with achlorhydria. J. Pharm. Pharmac. 26: 144 – 145, 1974.
22. Levine, R. R. factors affecting G. I. absorption of drug. Am. J. Dig. Dis. 15: 171- 188, 1970.
23. Nimmo, W. S. Drug diseases and altered gastric emptying. Clin. Pharmacok. 1: 189 – 203, 1976.
24. Baume, P. E., Nicholls, A. and Baxter, C. H., Inhibition of gastric acid
25. Secretion by a purified bacterial lippopolysaccharide. Nature, 215: 59 – 60, 1967.
26. Wyllie, I . H., Limbosch, I. M. and Nyhus, L. M. inhibition of gastric Secretion by bacterial lippopolysaccharide. Nature, 215: 879, 1967.
27. Leenon, F. H.H. and Van Miert, A. S. J. P. A. M. Inhibition of gastric Secretion by bacterial lippopoly saccharide in the rat. Europ. J. Pharmacol. 8: 228 – 231, 1969.
28. Van Miert, A. S. J. P. A. M. and De La Parra. Inhibition of gastric emptying by Endotoxin in conscious rats and modification of this response by drugs effecting the autonomic nervous system. Arch. Int. Pharmacodyn. 184: 27 – 33, 1970.
29. Parandota, J., Tillement, J. P., D' Athis, P.,Campos, H. and Barre, J. Binding of erythromycin base to human plasma proteins. Curr. Congr. Chemother. (11^{th}), 1: 671,1970.
28. Lashev, L. Pharmacokinetic research on amoxicillin in agricultural animals. Vet. Med. Nauki. 23 (10): 76 – 82, 1986.
29. Baggot, J. D., Principles of drug disposition in domestic animals. The Basic of Veterinary clinical pharmacology, pp. 144 – 218, W. B. Saunder & Co., Philadelphia. 1977.
30. Friis, C. and Ladefoged, O. Renal clearance of sulphathiazole in pigs with E. coli endotoxaemia. Zbl. Vet. Med. A. 26: 146 – 151, 1979.
31. Van Miert, A. S. J. P. A. M. Inhibition of gastric motility by Endotoxin (bacterial lippopolysaccharide) in conscious goats and modification of this response by drugs effecting the splanchnectomy, adrenalectomy, or adrenergic blocking agents. Arch. Int. pharmacodynam. 193: 405 – 414, 1971.
32. Riffat, S., Nawaz, M. and Rehman, Z. Pharmacokinetics of sulphadimidine in normal and febrile dogs. J. Vet. Pharmacol. Therap. 5: 131- 135, 1982.
33. Nawaz, M., Akhter, S. and Hashmi, A. S. Disposition kinetics and urinary excretion of sulphadimidine in normal and alloxan diabetic dogs. Acta. Pharmacol. Et Toxicol. 51: 63 – 68, 1982.
34. Nawaz M, Shah B H. Geonetical considerations in the quality assurance of pharmaceuticals. Proc. Int. Seminar on policies, management and quality assurance of pharmaceuticals. 1985;Apr 21-25 th, WHO and Ministry of health, Govt of Pakistan.
35. Ladefoged, O. pharmacokinetics of trimethprim (TMP) in normal and Febrile rabbits. Acta. Pharmacol. Et. Toxicol. 41: 507 – 514, 1977.