INT. J. BIOL. BIOTECH., 4 (2-3): 187-190, 2007.

IN VITRO DISTRIUBITON OF SULFADIAZINE IN WHOLE AND SKIMMED MILK OF GOAT

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ABSTRACT

The present study shows that sulfadiazine (SDZ) has greater capacity to bound with protein present in milk of goat. From this we calculated the percentage of drug in whole and skimmed milk. We also determined the amount of drug present in bound and free form. We also calculated the percentage of fat present in the milk samples. As the samples were collected from different rural areas of Faisalabad. We also determined the quality of the milk in these areas. The milk samples containing greater percentage of protein, showed the greater percentage of bound drug.

Key-words: Sulfadiazine, milk, goat, physiology, antibiotic.

INTRODUCTION

Sulphadiazine is effective against a wide range of microorganisms. It exhibits bactericidal action and is highly effective against several strains of bacteria that may be gram positive or gram negative such as *Enterobacteriacaeae* , including *E. coli* the Shigella and the Salmonella. Microorganisms that may be susceptible in vitro to sulfonamides include *Streptococcus pyogenes*, *Streptococcus pneumonia*, *Haemohilus influenzae* etc. (Goodman and Gilman, 2001).

Sulphadiazine is rapidly absorbed in the gut. About 70-100% of the oral dose is reabsorbed. It is about 50% plasma protein bound and 20-40% acetylated. Sulphadiazine sodium has good penetrability in the brain and is the preferred drug among the sulfonamides for meningitis, Acetylated derivatives are less soluble in urine. Sulphadiazine is excreted quite readily by the kidney, being acetylated approximately 50% percent of a single oral dose is eliminated within 24 hours and 60-85 percent within 48-72 hours. Sulfonamides is effective against a wide range of microorganisms.

Sulfonamides readily pass through the placenta and reach the fetal circulation. The concentration in the fetal circulation is enough to cause both antibacterial and toxic effects. Sulfonamides are eliminated from the body partly as the unchanged drug and partly as metabolic products. The largest fraction is extracted in the urine and the half life of sulfonamides in the body is thus dependent on renal function.

The value of goat milk in human nutrition has so far received very little factual and academic attention. Goats milk is believed to be more easily digestible and less allergenic than cow's milk. Goat milk protein form a softer curd which makes the protein more easily and readily digestible (Wood and Rebecca, 1988).

MATERIALS AND METHODS

The study was conducted to assess the distribution of sulphadiazine in whole and skimmed milk of goat. Ten milk samples were collected from healthy goats from different areas of Faisalabad. The predetermined amount of sulphadiazine was added in these samples. As the drug has protein binding capacity, the binding depends upon the total protein present in the milk. It is important to study the protein binding of drug because only unbound molecules was detected. *In vitro* distribution of the drug will be estimated in while and skimmed milk by colorimetric method (Brattan and Marshall, 1939). The data was analysed statistically (Steel *et al*., 1997).

ANALYTICAL METHOD

The concentration of sulfadiazine was determined by the method of Bratton and Marshall, (1939). After coupling violet color formed. The absorbance of this color was measured by spectrophotometer at 545 nm. Each sample was analyzed in triplicate.

PRINCIPLE OF THE METHOD

The two basic principles involved here are diazotization and coupling reactions. The diazotization reaction takes place in the presence of acid and sodium nitrite. The diazotization reaction is followed by coupling reaction, in the presence of coupling reagent. After the completion of coupling reaction, violet color was formed, which indicate the

presence of sulfonamides present in the samples.

Reagents used

The reagent used in this study were

1. Trichloroacetic acid 15%
2. Sodium nitrite 0.1%
3. Ammonium sulfamate 5%
4. Coupling reagent 0.1%
5. Sodium hydroxide 1N

Determination of standard factor

The standard factor was determined by dividing the concentration with the mean value of absorbance. A standard curve was formed by plotting concentration on Abcissa and absorbance on ordinate. Similarly, the milk samples to which (50 μg/mL) of sulfadiazine was added, were analyzed. Each whole milk and skimmed milk was treated in the same manner. After recording of absorbance, the amount of SDZ was calculated (Table 1).

Table 1. Absorbance of standard concentration of sulfadiazine in milk at 545nm.

Conc. (μg/mL)	Absorbance			Mean	Standard Conc./Abs	Factor
10	0.25	0.29	0.23	0.25	40	
20	0.342	0.356	0.340	0.342	58	
30	0.425	0.425	0.342	0.428	70	
40	0.489	0.498	0.509	0.500	80	
50	0.547	0.538	0.558	0.549	91	
60	0.580	0.586	0.590	0.588	102	

Mean value of standard factor = 73.5

Table 2. Percent binding of sulfadiazine with milk protein.

Sr. No.	pH of milk	% free drug	% bound drug
1	6.25	95.23	2.16
2	6.76	97.26	2.01
3	6.75	98.16	1.23
4	6.55	94.25	3.01
5	6.35	96.23	2.76
6	6.51	94.56	3.25
7	6.57	97.16	2.26
8	6.65	96.23	2.05
9	6.46	95.45	2.25
10	6.23	96.55	2.23
11	6.43	98.23	1.25
12	6.79	99.15	0.76
13	6.66	98.26	1.01
14	6.51	98.56	1.25
15	6.50	99.15	0.78
16	6.49	97.26	2.25
17	6.75	94.25	3.26
18	6.63	97.76	2.19
19	6.79	98.67	1.99
20	6.29	96.28	3.25
21	6.41	97.79	2.88
22	6.48	97.76	2.81
23	6.53	98.23	1.26
24	6.33	99.01	0.76
25	6.85	96.28	2.56
Mean	6.53	97.64	2.05
±SEM	0.23	3.65	0.29

RESULTS AND DISCUSSION

The pH of the sample ranged from 6.2-6.9 and averaged to 6.53±0.23. The percentage of drug that bound with milk protein ranged from 0.56 to 3.25 with Mean±SE 2.05±0.29 The percentage of free drug in the milk of goat was from 94.25-99.01 with Mean±SEM was 97.64±3.65 as given in the Table 2

The percentage of fat in goat milk sample ranged from 2-4 percent with Mean±SE was 3.46±0.163. The difference in between the values obtained from local and foreign data may be due to the differences between species, environmental conditions and also the difference in nutrition.

The percent distribution of sulfadiazine in whole milk with Mean±SE was 93.23±0.365. Similarly the calculation made for the percent distribution of sulfadiazine in skimmed milk with Mean±SE was 85.28 ±0.426. The percentage distribution of sulfadiazine in fat with Mean±SE was 4.71±0.145.These values are in table 3.

Table 3. Percent distribution of sulfadiazine in whole and skimmed milk

Sr. No.	% fat in milk	%age of drug in whole milk	%age of drug in skimmed milk	% of drug in fat
1	3.00	95.26	90.11	3.25
2	3.16	93.23	89.12	3.75
3	3.12	92.16	88.01	3.76
4	3.01	90.13	90.15	3.15
5	3.25	94.25	89.95	4.25
6	3.05	93.25	86.95	4.16
7	3.26	90.19	86.75	3.99
8	2.98	90.26	85.23	3.85
9	3.79	92.22	86.46	5.26
10	3.56	96.16	87.23	4.25
11	3.88	95.25	86.79	6.25
12	4.00	91.23	84.33	7.25
13	3.85	94.14	79.16	6.16
14	3.26	89.79	85.14	5.25
15	3.25	90.76	88.76	3.79
16	3.01	92.19	89.99	3.25
17	3.25	92.26	91.23	4.68
18	3.16	93.29	84.46	4.79
19	3.15	94.25	83.23	4.18
20	3.75	92.19	84.16	5.26
21	3.25	93.36	83.23	5.76
22	2.00	92.45	81.10	3.25
23	3.75	91.65	90.23	6.25
24	3.85	92.85	82.26	6.26
25	3.55	93.37	80.23	5.79
Mean	3.46	93.23	85.28	4.71
±SE	0.163	0.365	0.426	0.145

The present study dealt with *in vitro* distribution of sulfadiazine in whole and skimmed milk of goat. It was concluded that the %age distribution of the drug depends upon the amount of protein present in the whole milk. The greater the amount of protein present in the whole milk the greater the amount of drug present in the milk Soback and Lamminsivu (1979) studied the relationship between the protein binding of sulfonamides and their excretion in the milk. Sulfadiazine and sulfadimidine was given intravenously, to dairy cows the drugs were excreted into milk to greater extent, but the relationship becomes inverse in case of protein binding with milk protein.

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(*Accepted for publication July 2007*)