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Determination of Metabolite of Aspirin (Salicyluric Acid) by Colorimetric Method in Human Urine

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Abstract: Urinary excretion of Salicyluric acid (SUA) was investigated in twelve healthy male volunteers. Urine sample was collected up to 10 h. The concentration of salicyluric acid (SUA) a major metabolite of aspirin was measured colorimetrically. The concentration of salicyluric acid (SUA) (mean±SE) in urine was 200.5±14.9 μg mL^{-1}. Excretion of salicyluric acid was extremely variable and depends upon pH of urine. The results show that the mean±SE values for pH of the urine was 6.24±0.11, amount of drug excreted as salicyluric acid was 63.5±12 mg and percentage of dose excreted was 10.66±1.98. The cumulative amount of dose excreted as salicyluric acid was 191.3±58.2 mg and cumulative percent of dose excreted as salicyluric acid was 62.45±1.06. There is statistically slight variation in urinary excretion of SUA data when compared with foreign investigated values.

Key words: Salicyluric acid (SUA), urinary excretion, human volunteers, cumulative amount

INTRODUCTION

Aspirin (acetylsalicylic acid) occupies a unique place in medicine. Since its clinical introduction in 1899, we have becoming familiar with this drug and its many surprising effects, including reduced risk of cardiovascular diseases and possibly colorectal cancer, as well as its analgesic, anti-inflammatory and anti-platelet actions. Aspirin is thought to reduced risk of colorectal cancer, perhaps by as much as 40%, a property that is shared by other non-steroidal anti-inflammatory drugs (NSAIDS) (IARC, 1997; Paterson and Lawrence, 2001; Raymond, 2004).

Aspirin is the most commonly used therapeutic agent. Aspirin formerly referred to generically as acetylsalicylic acid is a member of the salicylate group of compounds comes from the bark of a willow tree and was used as a folk remedy for hundreds of years prior to what we know as modern day aspirin (Mayo Clinic, 2004). It is a non-steroidal anti-inflammatory drug (NSAID) that possesses analgesic, anti-inflammatory and antipyretic properties. Because of its efficacy, low toxicity and low cost it is esteemed as a standard with which all other non-steroidal anti-inflammatory agents are compared (Terry *et al*., 2004; Simon and Mills, 1980).

Aspirin may be used for the secondary prevention of myocardial infarction and stroke in patients with the history of such disorders. Aspirin irreversibly inhibits the platelets cyclo-oxygenase by acetylation, hence diminishes the formation of Thromboxane A_2 which is a powerful stimulator of platelets aggregation. So aspirin produces the anticoagulant effect by prolonging the bleeding time (Mayo Clinic, 2004; Deliargyris and Boudoulas, 2004; Loll *et al*., 1995).

Aspirin hydrolyzed in the stomach and in blood to salicylic acid and acetic acid, the biological half life is therefore only 20 min. The half life of salicylate is 2-4.5 h following the therapeutic doses but in overdose it increases to 18-36 h (Done, 1960). When the aspirin comes in circulation, liver and kidney generally take the major part in elimination but other tissues and organs also contribute their part such as lungs, blood, subcutaneous and mammary tissues (Glasgow, 1999; Punnon and Pekka, 1984).

Approximately 80% of small doses of salicylic acid is metabolized in the liver. Conjugation with glycin forms salicyluric acid and with glucuronic acid forms salicylic acyl and phenolic glucuronides. These metabolic pathways have only a limited capacity. Small amounts of salicylic acid are also hydroxylated to gentisic acid.

Salicylate are excreted mainly by the kidney as salicyluric acid (75%), free salicylic acid (10%), salicylic phenol (10%), acyl glucuronides (5%) and gentisic acid (<1%), when small doses (less than 250 mg in an adult) are ingested, all pathways proceed by first order kinetics, with an elimination half life of about 2-3 h. When higher doses of salicylate are ingested (more than 4 g), the half life becomes longer (15-30 h) (Hartwig-Otto, 1983).

The drugs being used in Pakistan for health programmes of human beings are imported from abroad either in raw or finished form. The studies carried out

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during the past decades under the local conditions have shown the difference between foreign and local species explained by an original term Geonetics, the geographical influence on genetics has been observed to be different biologically, physiologically and pharmacologically. The effect on drug metabolism, urinary excretion, blood protein and renal clearance has been observed to be different. These variations justify the description of therapeutic standards and dosage regimen on the basis of indigenous research (Nawaz, 1994; Nawaz *et al*., 1988).

Keeping in analysis, indigenous condition the present project was designed to study urinary excretion of salicyluric acid in male volunteers and also that the chemo preventive action of aspirin is due to primarily to its principle metabolite, salicyluric acid.

MATERIALS AND METHODS

Subjects: Twelve healthy young male volunteers having mean age 22.03 years, mean body weight 66.8 kg and mean height 171.66 cm selected from Chemistry Department (University of Agriculture, Faisalabad Pakistan) in 2003. All volunteers were apprised of the study protocol and a written consent was signed by each subject.

Study design: Sampling was done in month of December. Drug free urine sample was collected by each volunteer before the experimentation. After overnight fastening each volunteer received drug acetylsalicylic acid commercially known as Dispirin (soluble aspirin) in the dosage form of oral tablets 600 mg each, manufactured by Reckitt Bencikiser of Pakistan Ltd. Karachi and was purchased from local market. The drug was administered orally to twelve healthy male volunteers. Volunteers did not receive any medication seven days prior to and during course of study. All subjects were allowed to take breakfast 2 h after following the oral dose.

Salicyluric acid, 6 N Hydrochloric acid, Nitric acid (pure), Ferric nitrate (0.17%) and 1-2-dichloromethane were purchased from Merck, Germany. Spectral measurements were taken with Hitachi model U-2001 ultraviolet/visible Spectrophotometer (Japan) using 1 cm path length quartz cuvettes.

Collection of Urine samples and pH measurement: Urine samples were collected at 30, 60, 120, 180, 240 and 600 min after following oral dose. Total volume of urine voided during this time was noted. The pH of fresh urine samples were recorded with pH meter. The urine samples in plastic bottles were preserved in refrigerator at -20°C for further laboratory analysis.

Analytical method for the determination of salicyluric acid (SUA): Quantitative determination of acetylsalicylic acid as a free salicyluric acid (SUA) was carried out by a validated calorimetric method of Levy and Proknal as modified by Farid *et al*. (1975). The method was based on the selective extraction of free salicyluric acid (SUA) from 1 mL of urine by two 10 mL portions of CCl_4 after acidification with HCl. The re-extraction was made by adding 5 mL of 0.17% of Fe $(NO_3)_3$ solution (a 10-fold dilution of 17 g of Fe $(NO_3)_3$. $9H_2O$ in 1 L of 70 m mole L^{-1} HNO_3). The colored aqueous phase was centrifuged at 3000 rpm and absorbance was noted by spectrophotometer (Hitachi Model U-2001) at 530 nm. To calibrate the instrument run blank by adding 1 mL of distilled H_2O + 5 mL of 0.17% Fe $(NO_3)_3$ reagent.

Urinary excretion: Excretion is a process by which drug or its metabolites are eliminated from the body without chemical change.

Urinary excretion of drug was studied by calculating the following parameters:

Concentration = Standard factor×Absorbance

Urinary excretion

Amount of drug excreted = Uc×Uv

Where

Uc = Concentration of drug in urine (mg)
Uv = Total volume of urine voided

%age of dose excreted

$$\%\text{age of dose excereted} = \frac{\text{Amount of drug excereted (mg)}}{\text{Total dose of drug (given)}} \times 100$$

%age cumulative dose excreted

$$\%\text{age of cumulative dose excereted} = \frac{\text{Cumulative amount excereted (mg)}}{\text{Total dose of drug (given)}} \times 100$$

Statistical analysis: The results were tabulated and subjected to statistical calculation according to standard method. The results were given as mean±SE values (Muhammad, 2000).

RESULTS AND DISCUSSION

Metabolism and excretion constitute the elimination phenomenon. Human body is composed of physiologically and biochemically different characteristics

Table 1: pH of urine excreted after oral administration of (2×300 mg) aspirin

	Time (min)					
Volunteers	30	60	120	180	240	600
1	5.53	6.32	6.36	6.61	6.75	5.51
2	6.36	6.36	6.54	6.66	6.77	6.44
3	5.50	6.50	6.45	6.60	6.61	7.03
4	6.39	6.69	6.57	6.68	6.79	7.31
5	5.58	6.33	6.15	6.18	6.10	5.93
6	6.39	5.59	6.27	6.60	6.67	6.64
7	6.31	5.96	6.28	6.39	5.87	7.14
8	5.23	5.55	6.45	6.62	6.70	5.95
9	5.52	6.53	6.38	6.53	6.61	6.84
10	5.25	6.14	6.23	6.13	5.80	6.37
11	5.61	6.07	5.75	6.23	6.26	5.51
12	5.30	5.77	6.23	6.63	6.35	5.87
Mean	5.74	6.15	6.30	6.48	6.44	6.37
±SE	0.13	0.10	0.06	0.05	0.10	0.18

on the basis of age, body weight, sex, kidney functions which play an important role in drug elimination. Urinary excretion of drug depends upon pH of urine, dose of drug given and rate of urine flow.

Salicyluric acid (SUA) formed a lilac colored complex with iron (III) nitrate. The complex, which formed instantaneously at room temperature, was stable. The solution of the complex obeyed Beer's law at 530 nm, the wavelength of maximum absorption of radiation (λ_{max}) after an oral administration of 600 mg tablets.

Present study showed the mean±SE value for pH of urine voided by 12 healthy male volunteers after an oral administration of 2×300 mg dose was 6.24±0.11 in the range of 5.74 to 6.48 (Table 1). This value is comparable to mean±SE value for pH of urine 6.10±0.11 and ranged between 4.84 to 7.12 (Hanif *et al*., 2004) in twelve healthy female volunteers. This value can also be compare to report value for urine pH both for male and female volunteers at 900 mg orally administrated dose (Hutt *et al*., 1986). The reported value is 6.5±0.7 for males and 6.3±0.5 for female volunteers. The difference in the pH of urine may be due to sex variation, dose of drug used and nutritional ingredients.

Table 2 shows the mean±SE value for concentration of salicyluric acid in urine of 12 male volunteers is 200.5±14.9 μg mL^{-1} ranged from 115.34 to 685.8 μg mL^{-1} while in female volunteers this value is 225.8±53.8 μg mL^{-1} ranged between 8.6 to 234.81 μg mL^{-1} (Hanif *et al*., 2004) shows that the concentration of acetylsalicylic acid as salicyluric acid is higher in the urine of female volunteers than in male volunteers. The differences may be due to different environment and genetic factors. In most of cases the genetic make up of man and environmental conditions are different from their counter parts. The concentration of drug also depends upon pH of the fluid and pka of drug. pH is an important parameter which differs among local and foreign species (Nawaz, 1994).

Table 2 shows the amount of salicyluric acid (mg) excreted in urine of twelve male volunteers as Mean±SE value. The amount excreted was 63.5±11.7 mg in the range of 22.3 to 161.5 mg. While in female volunteers this value is 59.08±14.9 mg in the range of 0.95 to 245.7 mg (Hanif *et al*., 2004). That means the amount of acetylsalicylic excreted as salicyluric acid is lower in female volunteers than in male volunteers. The difference in the amount of salicyluric acid excreted is mainly due sex, pH of urine and other environmental factors may also affect.

Table 3 present the percent dose excreted as salicyluric acid in urine of twelve male volunteers following an oral administration of 2×300 mg aspirin was calculated as mean±SE value for percent dose of acetylsalicylic acid as salicyluric acid in 0-10 h excreted in urine of 12 male volunteers is 10.66±1.98% in the range of 0.29 to 26.91% while in female volunteers this value is 9.85±2.48% ranging from 0.16 to 40.90% (Hanif *et al*., 2004). This value is comparable to value for percentage of dose excreted as salicyluric acid (Montgomery and Sitar, 1986). The mean±SE value for percentage of dose excreted as salicyluric acid was 7.79± 2.7. The difference in the % dose may be due to sex variation, dose, chemical nature of drug as two different formulations are used above and different environmental conditions.

Table 4 shows the cumulative amount of acetylsalicylic acid excreted as salicylic acid (mg mL^{-1}) in urine of twelve healthy male volunteers the mean±SE value for cumulative amount of acetylsalicylic acid excreted as salicyluric acid (mg) in urine of 12 male volunteers is 190.9±58.2 mg in the range of 0.59 to 401.93 mg. This value is comparable to mean±SE value for cumulative amount in females reported 354.53±7.15 mg in the range of 0.61 to 384.1 mg (Hanif *et al*., 2004) and this study shows that the cumulative amount of acetylsalicylic acid as salicyluric acid excreted in female volunteers is higher than males. This difference may be due to sex variation. Table 4 shows the cumulative percent amount of acetylsalicylic acid as salicyluric acid (mg mL^{-1}) in urine of twelve male volunteers at different time intervals following oral administration of 2×300 mg aspirin. The mean cumulative percent doe excreted as salicyluric acid in the present study is 62.45±1.06% and the range is 0.09 to 66.99%. This value is comparable to the cumulative percent amount of acetylsalicylic acid excreted as salicyluric acid in 0-10 h (59.08±1.1%) in the range of 0.1 to 64.03% reported by Hanif *et al*. (2004) in female volunteers. This value is also comparable to value (Hutt *et al*., 1986, 1982). They studied the metabolism of

Table 2: Concentration and amount of salicyluric acid excreted after oral administration of (2×300 mg) aspirin

	Time (min)											
	30		60		120		180		240		600	
Volunteers	Conc. of SUA (μg mL^{-1})	Amount of SUA (mg mL^{-1})	Conc. of SUA (μg mL^{-1})	Amount of SUA (mg mL^{-1})	Conc. of SUA (μg mL^{-1})	Amount of SUA (mg mL^{-1})	Conc. of SUA (μg mL^{-1})	Amount of SUA (mg mL^{-1})	Conc. of SUA (μg mL^{-1})	Amount of SUA (mg mL^{-1})	Conc. of SUA (μg mL^{-1})	Amount of SUA (mg mL^{-1})
1	140.27	53.30	151.96	24.31	163.65	93.28	187.02	130.92	144.17	37.48	171.45	22.28
2	137.16	20.57	176.90	45.11	194.43	128.58	197.17	102.52	210.41	35.77	194.83	68.19
3	132.48	9.27	142.61	29.94	148.07	72.55	163.65	81.01	180.02	99.01	190.15	51.34
4	136.38	16.36	151.96	41.03	171.42	108.01	175.34	105.02	173.79	86.89	333.55	76.71
5	140.27	50.50	152.74	47.35	136.38	96.83	163.65	58.91	199.50	33.91	428.63	109.31
6	179.24	9.58	169.11	28.74	115.34	92.27	685.81	120.01	317.18	87.22	173.79	46.92
7	164.43	35.35	132.48	18.54	154.30	86.45	163.65	50.73	202.62	48.68	436.42	161.47
8	450.45	22.52	176.90	1.76	217.43	78.27	126.25	113.62	123.13	110.82	155.86	32.73
9	236.13	10.62	190.93	30.54	216.65	103.99	202.62	92.19	194.83	59.42	233.79	72.47
10	208.86	21.93	128.58	30.86	132.48	70.21	194.83	80.85	243.15	80.27	420.83	117.93
11	163.65	24.54	296.15	102.97	132.48	18.54	175.34	115.73	195.61	72.37	246.26	44.32
12	144.16	7.92	206.52	7.22	164.43	112.64	159.76	121.41	243.93	51.22	174.56	43.64
Mean	186.1	23.54	173.1	34.03	162.2	88.43	216.3	97.74	202.2	66.92	263.3	70.6
±SE	25.8	4.46	13.1	7.42	9.55	8.03	43.1	7.35	14.5	7.56	31.9	11.7

Table 3: % age dose of salicyluric acid (mg mL^{-1}) excreted after oral administration of (2×300 mg) aspirin

	Time (min)					
Volunteers	30	60	120	180	240	600
1	8.88	4.05	15.54	21.82	6.24	3.71
2	3.42	7.51	21.43	17.08	5.96	11.36
3	1.54	4.99	12.09	13.50	16.50	8.55
4	2.72	6.83	18.0	17.53	14.48	12.86
5	8.41	7.89	16.13	9.81	5.65	18.21
6	1.64	4.79	15.37	20.0	14.53	7.82
7	7.89	3.09	14.40	8.45	8.1	26.91
8	3.75	0.29	13.04	18.93	18.47	5.45
9	1.77	5.09	17.33	15.36	9.90	12.07
10	3.65	5.14	11.70	13.47	13.37	19.6
11	4.09	17.02	3.09	19.28	12.06	7.38
12	1.32	1.20	18.77	20.23	8.53	7.27
Mean	4.09	5.66	14.74	16.29	11.15	11.77
±SE	0.79	1.23	1.34	1.22	1.26	1.95

Table 4: Cumulative and % cumulative dose of salicyluric acid excreted after oral administration of (2×300 mg) aspirin

	Time (min)											
	30		60		120		180		240		600	
Volunteers	Cumulative (mg)	% Cumulative (mg mL^{-1})	Cumulative (mg)	% Cumulative (mg mL^{-1})	Cumulative (mg)	% Cumulative (mg mL^{-1})	Cumulative (mg)	% Cumulative (mg mL^{-1})	Cumulative (mg)	% Cumulative (mg mL^{-1})	Cumulative (mg)	% Cumulative (mg mL^{-1})
1	53.30	8.88	77.62	12.93	170.90	28.48	301.83	50.30	339.32	56.55	361.60	60.24
2	20.57	3.42	65.68	10.94	194.27	32.37	296.83	49.46	332.57	55.42	400.76	66.79
3	9.27	1.54	39.22	6.53	111.77	18.62	192.79	32.13	291.80	48.63	343.14	57.19
4	16.36	2.72	57.39	9.56	165.41	27.56	270.62	45.10	357.51	59.58	357.51	59.58
5	50.50	8.41	97.85	16.30	194.68	32.44	253.60	42.26	287.51	47.91	396.81	66.13
6	9.85	1.64	38.60	6.43	130.88	21.81	250.89	41.81	338.14	56.35	385.04	64.17
7	35.35	5.89	53.90	8.94	140.31	23.38	191.04	31.84	239.67	39.94	401.15	66.85
8	22.52	3.75	24.29	4.04	102.56	17.09	216.19	36.03	327.01	54.50	359.74	59.95
9	10.62	1.77	41.17	6.86	145.16	24.19	237.36	39.56	296.78	49.46	369.26	61.54
10	21.93	3.65	52.79	8.79	123.00	20.50	203.86	33.97	284.10	47.35	401.93	66.99
11	24.54	4.09	0.59	0.09	145.26	24.21	260.99	43.49	333.37	55.56	377.70	62.59
12	7.29	1.32	15.15	2.52	127.79	21.29	249.21	41.53	300.44	50.04	344.08	57.34
Mean	23.51	3.93	47.02	7.83	146.00	24.33	243.8	40.62	310.69	51.77	374.89	62.45
±SE	4.47	0.74	7.74	1.29	8.65	1.44	10.7	1.79	9.52	1.59	6.38	1.06

aspirin in man and observed that major urinary metabolite was salicyluric acid 56-68% of dose. The elimination of this metabolite ranged from 19.8 to 65% of the dose. The cumulative percent amount is higher in females than in males after oral dose of 2×300 mg aspirin. This difference may be due to sex variation, fluctuation in urine pH, environmental conditions and nutritional ingredients (Nawaz, 1994).

There is contradiction observed in our values and foreign investigated values. This difference may affect the chemical nature and pharmacokinetic of acetylsalicylic acid (aspirin) in our local environmental conditions. The study supports the comprehensive evaluation of the drug under indigenous conditions to obtain the useful information on which the rational dose regimens of drug could be based.

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