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Original Research Paper

Zoology

EFFECT OF SALICYLATE ON ALBINO RATS BY STUDY OF SERUM GLOBULIN PATTERN

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ABSTRACT Salicylate is widely used as an analgesic in medicine. It has been observed that many of the deadly effigies of salicylate have resulted in the human body. Therefore, the persentant study was designed to evaluate if there is any change in albumin protein in serum after liver damage due to salicylate treatment, if there is a compensatory binding reaction and the other in serum for salicylate treatment model. The young albino rats of Vistar Strain, weighing between 180 and 200 grams were selected for experimental work, were taken and fed a standard diet for two weeks. After this, they were divided into 3 groups and treated with sodium salicylate for three weeks and six weeks, respectively. After treatment with salicylate for three weeks and six weeks, the body weight of the rats had decreased; after treatment with sodium salicylate, the reason for the reduction in body weight could be the reason. Albumin was slightly reduced, because after 3 weeks of salicylate treatment, beta-globulin had slightly increased. Albumin was significantly reduced and after 6 weeks of salicylic treatment, alpha-1 and beta-1 globulin significantly increased. There is no significant change in the other globulin fraction after 6 weeks of salicylate treatment. There is a significant gradual reduction in the transferrin globulin in rats treated with salicylate, as compared to that which inhibits the ion transport mechanism of ions from Fe ions.

KEYWORDS : Salicylate, albino rats and Globulin

INTRODUCTION

Salicylic acid anti-inflammatory agents, including acetylsalicylic acid (aspirin) and salts of salicylic acid (1), improve sensitivity to animal insulin (2-4) With improvements in type 2 diabetes (7-8). The mechanism of insulin sensitization is uncertain and may include the blocking of inhibition of inb-kenase-itor and, therefore, the interference with the activity of the effect of atomic factors (9, 10) and / or proteins. of phosphorylation and union of the improvement of CCAAT (A transcription factor, included in the metabolic and inflammatory pathways) (11,12), with associated reduction in the inflammatory cytokines.

The 11-hydroxysteroid dehydrogenase type 1 (11 HS-HSD1) induces the appearance of active cortisol of the inert courtesan (11-dihidocarcostone to the cardiocortostorone), in which the liver and adipose tissue are involved (13). The glucocorticoid action and insulin resistance increase in genetically modified mice, adipose tissue (11) or overexposure of 11 HS-HSD1 in the liver (15), while high-fat foods contain resistance to Insulin and central obesity against Hsd11b1 Directed disruption (16-16) 17). Medical inhibition of 11 -HSD1 reduces intracellular glucocorticoid levels and, therefore, increases insulin sensitivity in rodents and humans (18-19). Circumstances show that salicylates can reduce 11-HS-HSD1 in adipose tissue. Updates of 1121-HSD1 in culture, including human preadipocytes (20-21) in many cell types, including proinflammatory cytokines, tumor necrosis factor (TNF) -α, interleukin-1le and interleukin-6. In human obesity, systemic and intra-adipose inflammation (10), mRNA levels of 11 -HSD1 and activity decrease in the liver (22,23), but subcutaneous adipose tissue has increased (24,23,25).) 26) and decreases the elevation of intra-adipose cortisol (29) in visceral adipose tissue (27,28). These observations increase the likelihood that expression of Intra-Adipose 11rα-HSD1 increases in obesity due to the proliflamato status. In fact, in the lipidiastrophy of HIV, the intraadipient inflammation pattern increases, the intra-adipose and systemic 11-HSD1 activity (30).

Present study therefore was designed to evaluate whether there is any change in albumin pattern in serum after the damage to liver due to the treatment of salicylate.

MATERIALS AND METHODS

For the experimental work, young albino rats of around 175-200 grams of weighing per strain were selected. Before experimenting, the rats were deposited under laboratory conditions for 2 weeks. They were fed with standard diet having the following composition.

Composition of standard diet (Per 100 gms)

Constituents	Weight in gms
Carbohydrate (Wheat flour)	73.0
Protein (Gram flour)	15.0
Cellulose	4.5
Multi Vitamins	2-3 Drops
Vegetable Oil	1 teaspoon
Salt Mixture	6.0
(Hubbel Mendel Walkman)	

Composition of salt mixture (Hubbel & Walkman, 1973)

Salts	Quantity in gms
$CaCo_3$	543.0
$MgCo_3$	25.0
$MgSo_4$	16.0
NaCl	69.0
KCl	112.0
Kh_2Po_4	212.00
KI	0.80
$MnSo_4$	0.35
NaF	1.00
$Al2(So_4)_3$	0.17
$CuSo_4$	0.90

MAINTENANCE & ANIMAL CARE

After 2 weeks of acclimatization under laboratory conditions, the rats were divided into two groups. These groups were kept in separate cages under controlled habitual conditions with good sunlight, air and healthy conditions. Cages and water bottles were cleaned at regular intervals.

Grouping of Rats

All the 18 rats were divided into 2 groups, one group containing 6 rats and the other containing 12 rats. They were weighed weekly and record was maintained of each week.

Group No. 1

This group of 6 rats was the controlled one and was treated

with standard diet throughout the period.

Group No.2 (Experimental)

The 12 rats in this group were fed with sodium salicylate with standard diet for 3 and 6 weeks. Sodium salicylate was orally administered in the form of 200 mg per kg. Body weight of the rat per day (Davison, 1971). Then, this group was divided again into 2 groups and each group had 6 mice.

Group A this group of 6 rats that were fed with sodium salicylate for 3 weeks and sacrificed after 3 weeks of treatment.

Group B this group of 6 rats that were fed with sodium salicylate for 6 weeks and sacrificed after 6 weeks of treatment.

Preparation of Samples

After the treatment the final weight of rats were recorded and they were sacrificed. Blood was taken from the heart by syringe and allowed to clot. A clear liquid (Serum) excludes from the clotted blood through centrifugation at 3000 rpm for 10 min. Serum was stored at 0^0 to 4^0C.

Electrophoretic Separation of Serum Proteins on Polyacrylamide Gel

Gel Preparation

Followings solutions were prepared:

a) Solution A was prepared with 11.3 gm of Acrylamide & 0.3 gms of Bis-Acrylamide in 50 ml of distilled water.
b) Solution B was prepared with 12 ml. of IN-HCL, 0.2 ml. of Temed & 8.575 gms of Tris, Final volume was adjusted to 50 ml with distilled water.
c) Solution C was prepared with 0.15 gms of Ammonium per sulphate in distilled water. Final volume was adjusted to 50 ml with. This solution was always prepared freshly.

Casting the Gel:

Plates, spacers and template were cleaned and dried thoroughly. Plates with spacers using cello-tape & clips were fixed. Solution A, B and C were mixed in a beaker and poured in between the plates. Template was placed in incubator having temperature below 15^0C. Mixing the solution and casting the gel was done below 20^0C. The gel for polymerization was kept in cold room for about three hours.

The composition of running buffer was as follows:

STOCK BUFFER:- was prepared with 7.2 gms glycine & 1.5 gms of Tris. Final volume was adjusted 500ml with distilled water.

RUNNING BUFFER:- was prepared with one part of stock buffer & 9 part of distilled water. pH was adjusted to 8.6.

Loading The Sample

Before loading the sample a drop of tracking dye (0.1% Bromophenol blue & 68.4 gms sucrose in 100 ml of distilled water) was placed on the gel. 20 mu l of serum was loaded with the help of micropipette.

The running buffer was filled both on the upper and lower chamber and constant voltage current was applied. The sample was first run at 150 Volts for half an hour and was increased to 250 volts until the tracking dye reached the end.

Visualisation of Gels

The gels with serum protein sample separated were stained in coomasie brilliant blue (C.B.B.) (C.B.B.: 1.25 gms, Methanol 230ml, Acetic acid 40ml., raise upto 500ml with distilled water) for 10 minutes. The gel were transferred in destainer (Methanol, Acetic acid and distilled water in 6:1:6 ratio). After sufficient destaining the bands were observed.

QUANTITATIVE ANALYSIS

After the localization of the protein bands, they were eluted. First gel was placed in a plate and cut into very fine piece with the help of a sharp blade, pieces were transferred into a test tube and covered with aluminium foil. Next day the contents of test tubes were spinned at 3000 rpm, for 10 minutes supernatant was transferred into the test tube and O.D. was taken at 540nm.

Relative mobility (Rm or Rf Value) determination:

Distance travelled by protein and tracking dye was calculated for determination of RF value.

FORMULA USED: $\frac{\text{Distance travelled by Protein}}{\text{Distance travelled by tracking dye}}$

Preparation of Working Standard

Stock solution of protein was prepared by dissolving 10 mg of Albumin in 100ml of Tris glycine buffer. After that 25mg, 30mg, 40mg, 50mg, of B.S.A. was taken and dissolved in 100 ml of Tris Glycine buffer. This working was taken and graph of standard was plotted.

Preparation of Blank

Reagent Blanks was prepared by mixing 2 ul C.B.B. stain & ml of Tris glycine buffer.

Determination of unknown concentration

Graph of standard was plotted against the O.D. and the concentration after that O.D. of unknown sample was pointed on the standard and the concentration was determined in mg/100ml.

RESULTS AND DISCUSSION:

BODY WEIGHT

The body weights of rats in experimental group are summarised in Table-1. It is seen that there increasing body weight in the group of controlled rats. However a slight decrease in body weight in the group of rats fed with Sodium Salicylate for 3 Weeks and a significant decrease was noted in body weight in the group of rats fed with Sodium Salicylate for 6 weeks.

Serum Globulins

A quantitative analysis of serum globulins (fig-3) indicates that fast moving bands on slab get which corresponds to albumin followed by alpha, beta, gamma and transferrin globulins. In serum of salicylate treated rats aplpha-1 band is prominent.

A quantitative estimation of globulin in control and after 3 weeks and 6 weeks of salicylate treated Rate summarized in Table 2 and Fig.2. Albumin slightly decreases and beta-1 globulin slightly increases after salicylate treatment for 3 weeks.

In salicylate treated Rates for 6 weeks, Albumin significantly decreases and beta-1 globulin significantly increases. There is no significant alternation in other globulin fractions.

Table.1: Body Weight of Rate after treatment duration in week

Group-A (Control)	Group-B Salicylate for 3 weeks	Group-C Salicylate for 6 weeks
195	200	213
197	200	209
199	195	205
203	194	196
205	190	192
212	188	190

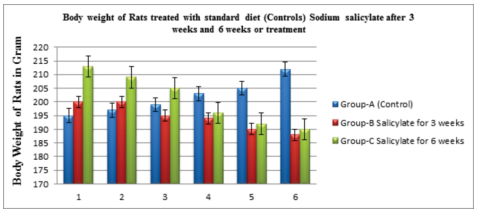


Fig. 1: Body weight of Rats treated with standard dite.

Table 2 Serum Globulin Concentration in Control and Treated Rats

Group of Rats	Albumin	1	2	1	2		Transferrin
Group A Control	2.8 0.33 P<0.05	0.81 0.50 P<0.05	0.92 0.51 P<0.05	0.79 0.40 P<0.05	0.45 0.32 P<0.05	0.85 0.33 P<0.05	0.38 0.14 P<0.05
Group B Salicylate for 3 week	0.3 0.08 P<0.05	3.9 0.14 P<0.05	0.85 0.33 P<0.05	1.5 0.41 P<0.05	Trace	0.85 0.33 P<0.05	Trace
Group C Salicylate for 6 week	Trace	5.3 0.08 P<0.05	0.65 0.01 P<0.05	2.3 0.08 P<0.05	Trace	0.98 0.01 P<0.05	Trace

Concentration In Grams/100ml

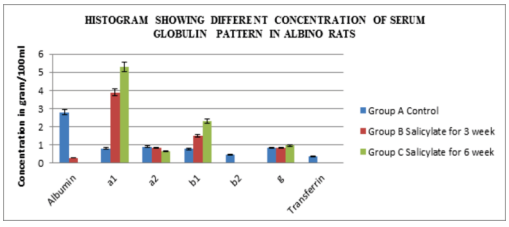
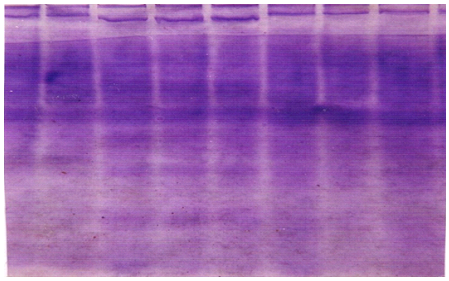


Fig.2: Histogram Showing Different Concentration Of Serum Globulin Pattern In Albino Rats

Table 3 RF Values for different groups of Rats

Group of Rats	Total length of Gel (M)	Dye Position (m)	Band Position (m)	Mean band (m)	RF Value	Conc. Gm/100ml.
Group A (Control)	16	10.5	1.6-1.7 1.9-2.0 2.3-2.6 3.0-3.4 4.1-4.2 5.5-5.6 6.8-7.0	1.65 1.95 2.4 3.2 4.15 5.75 6.9	0.15 0.18 0.22 0.30 0.39 0.54 0.65	0.33 0.81 0.92 0.79 0.45 0.85 0.38
Group B Salicylate for 3 week	16	10.5	1.5-1.6 2.1-2.2 4.0-4.1 5.2-5.3 6.5-6.6	1.55 2.15 4.05 5.25 6.55	0.15 0.20 0.38 0.50 0.62	0.38 0.14 0.33 0.41 0.85
Group C Salicylate for 6 week	16	10.5	1.7-1.8 2.3-2.4 3.0-3.2 5.2-5.3	1.75 2.35 3.1 5.25	0.16 0.22 0.29 0.5	5.3 0.65 2.3 0.98

Salicylate For 6 weeksSalicylate For 3 weeksControl



Electrophoretic separation of serum Globulin in control and salicylate Treated (three weeks and six weeks) albino rats.

DISCUSSION:

Neiderland (1963) explained the action of salicylate on the nitrogen balance, which results in to aminoaciduria by activation of corticosteroid, that increases protein catabolism. This may be the cause of the decrease in body weight in rats after salicylic treatment in the present work.

Administration of salicylate develops acute liver injury or chronic hepatitis with rabbits in connective tissue disorder (25). Such degenerative changes in liver by salicylate were also noted in Salicylate Poisoning In man(28) in Rabbits (Janota, 1960), Netherlands, 1963 (26), they suggested that it is due to interference in the removal of fat from or metabolism of fat by liver. The degenerative activity of the hepatocytes leads to the regenerative activity of the cells, which is the most salicylate treated animal in which the hepato-cellular damage is maximum (18), it is also observed that these necrotic changes in the liver may be due to a non-united form. Fatty acids are caused by the rational displacement of salicylate in place of the albumin bound to salicylate. This is the reason for the disappearance of albumin in serum after salicylic treatment. Liver function and other tissue failures generally result in lower albumin levels, resulting in increased production of other globulins. Explain the increase in alpha-1 globulin and beta-1 globulin in rats treated with salicylate.

The increase of alpha-1 globulin by salicylate can be correlated by the regenerative activity of liver cells.

These observation not only identifies rapid decrease albumin production by liver in salicylate treated Rats; it is also significant to note that salicylate doesn't bring about any change in gamma globulin. It seems that salicylate doesn't alter immune state of the Rat.

There is significant gradual reduction in Transferrin globulin in salicylate treated Rats as compared to that in control. There are several reports where salicylate disturbs hematopoietic mechanism.

REFERENCES:

1. Rainsford KD, Buchanan WW. Aspirin versus the non-acetylated salicylates. Baillieres Clin Rheumatol1990;4:247–268 ..1
2. Kim JK, Kim YJ, Fillmore JJ, Prevention of fat-induced insulin resistance by salicylate. J Clin Invest2001;108:437–446 ..2
3. Yuan M, Konstantopoulos N, Lee J, Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science 2001;293:1673–1677 ..3
4. Barreiro GC, Prattali RR, Caliseo CT, Aspirin inhibits serine phosphorylation of IRS-1 in muscle and adipose tissue of septic rats. Biochem Biophys Res Commun 2004;320:992–997 ..5
5. González-Ortiz M, Martínez-Abundis E, Balcázar-Muñoz BR, Robles-Cervantes JA. Inhibition of cyclooxygenase-1 or -2 on insulin sensitivity in healthy subjects. Horm Metab Res 2001; 33:250–53 ..6
6. Koska J, Ortega E, Bunt JC. The effect of salsalate on insulin action and glucose tolerance in obese non-diabetic patients: results of a randomised double-blind placebo-controlled study. Diabetologia2009;52:385 ..9
7. Baron SH. Salicylates as hypoglycemic agents. Diabetes Care 1982;5:64–71 ..10
8. Goldfine AB, Fonseca V, Jablonski KA, Pyle L, Staten MA, Shoelson SE; TINSAL-T2D (Targeting Inflammation Using Salsalate in Type 2 Diabetes) Study Team. The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. Ann Intern Med 2010;152:346–357 ..12
9. Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science 1994;265:956–959 ..13
10. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006;116:1793–1801 ..14 ..14
11. Cieslik KA, Zhu Y, Shtivelband M, Wu KK. Inhibition of p90 ribosomal S6 kinase-mediated CCAAT/enhancer-binding protein beta activation and cyclooxygenase-2 expression by salicylate. J Biol Chem 2005;280:18411–18417 ..15
12. Wu KK. Transcription-based COX-2 inhibition: a therapeutic strategy. Thromb Haemost 2006;96:417–422 ..16
13. Seckl JR, Walker BR. Minireview: 11beta-hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action. Endocrinology 2001;142:1371–1376 ..17
14. Masuzaki H, Paterson J, Shinyama H. A transgenic model of visceral obesity and the metabolic syndrome. Science 2001;294:2166–2170 ..18
15. Paterson JM, Morton NM, Fievet C. Metabolic syndrome without obesity: Hepatic overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in transgenic mice. Proc Natl Acad Sci U S A 2004;101:7088–7093 ..19
16. Kotelevtsev Y, Holmes MC, Burchell A. 11beta-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses

and resist hyperglycemia on obesity or stress.Proc Natl Acad Sci U S A 1997;94:14924–14929 ..20
17. Morton NM, Paterson JM, Masuzaki H. Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11 beta-hydroxysteroid dehydrogenase type 1-deficient mice. Diabetes2004;53:931–938. ..22
18. Walker BR, Connacher AA, Lindsay RM, Webb DJ, Edwards CR. Carbenoxolone increases hepatic insulin sensitivity in man: a novel role for 11-oxosteroid reductase in enhancing glucocorticoid receptor activation. J Clin Endocrinol Metab 1995;80:3155–3159. ..23
19. Rosenstock J, Banarer S, Fonseca VA. INCB13739-202 Principal Investigators. The 11-beta-hydroxysteroid dehydrogenase type 1 inhibitor INCB13739 improves hyperglycemia in patients with type 2 diabetes inadequately controlled by metformin monotherapy. Diabetes Care 2010;33:1516–1522. ..26
20. Tomlinson JW, Moore J, Cooper MS, et al. Regulation of expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue: tissue-specific induction by cytokines. Endocrinology2001;142:1982–1989. ..27
21. Balachandran A, Guan H, Sellan M, van Uum S, Yang K. Insulin and dexamethasone dynamically regulate adipocyte 11beta-hydroxysteroid dehydrogenase type 1. Endocrinology 2008;149:4069–4079. ..29
22. Stewart PM, Boulton A, Kumar S, Clark PM, Shackleton CH. Cortisol metabolism in human obesity: impaired cortisone—>cortisol conversion in subjects with central adiposity. J Clin Endocrinol Metab1999;84:1022–1027. ..30
23. Rask E, Olsson T, Söderberg S. Tissue-specific dysregulation of cortisol metabolism in human obesity. J Clin Endocrinol Metab 2001;86:1418–1421. ..31
24. Livingstone DE, Jones GC, Smith K. Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. Endocrinology 2000;141:560–563. ..32
25. Wake DJ, Rask E, Livingstone DE, Söderberg S, Olsson T, Walker BR. Local and systemic impact of transcriptional up-regulation of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue in human obesity. J Clin Endocrinol Metab 2003;88:3983–3988. ..33
26. Walker BR. Extra-adrenal regeneration of glucocorticoids by 11beta-hydroxysteroid dehydrogenase type 1: physiological regulator and pharmacological target for energy partitioning. Proc Nutr Soc2007;66:1–8. Baudrand R, Carvajal CA, Riquelme A, et al. Overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in hepatic and visceral adipose tissue is associated with metabolic disorders in morbidly obese patients. Obes Surg 2010;20:77–83 ..34
27. Veilleux A, Rhéaume C, Daris M, Luu-The V, Tchernof A. Omental adipose tissue type 1 11 beta-hydroxysteroid dehydrogenase oxoreductase activity, body fat distribution, and metabolic alterations in women. J Clin Endocrinol Metab 2009;94:3550–3557. ..35
28. Sandeep TC, Andrew R, Homer NZ, Andrews RC, Smith K, Walker BR. Increased in vivo regeneration of cortisol in adipose tissue in human obesity and effects of the 11beta-hydroxysteroid dehydrogenase type 1 inhibitor carbenoxolone. Diabetes 2005;54:872–879. ..36
29. Sutinen J, Kannisto K, Korsheninnikova E, et al. In the lipodystrophy associated with highly active antiretroviral therapy, pseudo-Cushing's syndrome is associated with increased regeneration of cortisol by 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue. Diabetologia 2004;47:1668–1671. ..37
30. Wabitsch M, Brenner RE, Melzner I. Characterization of a human preadipocyte cell strain with high capacity for adipose differentiation. Int J Obes Relat Metab Disord 2001;25:8–15. ..38