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THE EFFECT OF ACETYLSALICYLIC ACID ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN FEMALE ALBINO RATS

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ABSTRACT : The present investigation is aiming at studying the effect of oral administration of aspirin (acetylsalicylic acid, ASA) drug on female albino rat, *Rattus rattus norvegicus*. The female rats (n=24) were allocated into 2 groups as control and treated. The treated rats (n=12) were given orally with high dose of aspirin at a dose of 100 mg/ kg body weight for 15 days (n=6) and 30 days (n=6). And body weight, relative organ weights, hematological parameters such as Hb %, total count RBC and total count WBC; and biochemical estimations in blood serum such as glucose, protein, cholesterol and calcium were measured. The aspirin did not showed much significant variations in the body weight, relative organ weights, serum protein, serum cholesterol, serum calcium, total counts RBC and Hb %; while, aspirin declined serum glucose level and elevated total count of WBC. The present investigation indicated that the aspirin impairs serum glucose level and total count of WBC by modulating of certain enzymes metabolism in albino rats.

Key Words: Aspirin (acetylsalicylic acid, ASA), body weight, relative organ weight, hematological parameters, biochemical estimations, *Rattus rattus norvegicus*.

INTRODUCTION

Since the early days of ancient and modern medical field, aspirin (acetylsalicylic acid, ASA) has been used as a one of the most famous, cheapest, easily available and widely used Non Steroidal Anti Inflammatory Drug (NSAID). The uses of aspirin such as, anti- inflammatory (in joint diseases), anti-platelets (in cardiovascular disease), analgesic and antipyretic (Adnan, *et al.,* 2005). Aspirin is rapidly absorbed from the stomach and small intestine, primarily by passive diffusion across the gastrointestinal (GI) tract and rapidly hydrolyzed to salicylic acid by esterases in the GI mucosa and plasma. Salicylic acid is widely distributed throughout the body, with the highest concentrations in the plasma, liver, renal cortex, heart, and lungs. It is metabolized via conjugation in the liver to form salicyluric acid and several other metabolites (Marcia, 2007).

A recent study demonstrated that, ASA altered plasma thyroid hormone levels and also attenuated the stress-induced cortisol response to stress in tilapia (*Oreochromis mossambicus*) (Anholt, *et al*., 2003). A large dose of salicylate stimulates corticosteroid secretion by the adrenal cortex. In connection to this, aspirin reduces the level of corticosterone and T_3 hormone that consequently declined serum cholesterol and glucose metabolism (Mohammed, *et al*., 2010).

Recently, some studies have also shown that, long term use of aspirin may decrease the risk of colorectal neoplasia and the progression of certain cancers, due to prostaglandins play an important role in maintaining blood supply to tumors and inhibition of their production by aspirin will therefore limits tumor growth (Sandler, *et al*., 2003). As well as, various workers have reported that the high doses of aspirin caused death of the blood vessel cells (Dikshit, *et al*., 2006).

Aspirin can also have adverse effects in pregnancy as, increases the duration of pregnancy, the mean duration of spontaneous labor, perinatal blood loss, increased risk of stillbirth and fetal growth retardation (Collins and Turner, 1975). Aspirin can interact with a number of other medications like; coumadin (warfarin) is a blood thinner that could cause life threatening hemorrhage in combination with aspirin (Joe, *et al.,* 1993). Aspirin treated guinea pigs increases the production of blood eosinophils and possibly basophiles (Hirai, *et al*., 2001). Besides this, aspirin when injected into the animal significantly aggravated deformability of erythrocytes (Korbut, 2003).

Aim of the work

The present investigation aims to study the effect of oral dose of aspirin (acetylsalicylic acid, ASA) (100 mg/ kg body weight) on body weight, haematological parameters *i.e.* Hb %, Total Count-RBC (TC-RBC) and Total Count-WBC (TC-WBC) along with biochemical estimations *i.e* protein, glucose, cholesterol and calcium levels in blood serum of female albino rat, *Rattus rattus* were done after 15 and 30 days.

MATERIALS AND METHODS

Material

Aspirin (acetylsalicylic acid) tablets containing (75 mg/tablet) as anti-inflammatory drug was purchased from the market, trade name Ecospirin-75 and manufactured by USV LTD, B.S.D. Marg, Govandi, Mumbai-400088. Tablets were powdered gently and given orally to the experimental animals at a dose of 100 mg/kg body weight.

Experimental animals

The above study was carried out on 18 adult female albino rats (body weight ranging between 150-200 gm). The animals were acclimatized to laboratory condition *i.e.* 23 ± 2ºC temperature with 14 hours light and 10 hours dark cycle at least for 7 days prior to initiating to the experiment. The animals were fed with Gold Mohar rat feed and water *ad libitum*. A dose of aspirin (100mg/kg b. wt.) was daily administered orally, through gavage (the administration of food or drugs by force, especially to an animal) for 15 and 30 days. All animals were divided into 3 groups of 6 each.

Group 1: Fed with normal diet and water *ad libitum* served as control.

Group 2: Fed with normal diet and received aspirin orally (100mg/kg b. wt.) through gavage for 15 days.

Group 3: Fed with normal diet and received aspirin orally (100mg/kg b. wt.) through gavage for 30 days.

After above duration, the animals were weighed, sacrificed on 16th and 31st day and blood samples were collected through cardiac puncture, serum were separated and following parameters were done by appropriate methodology.

Parameters:

A. **Body weight and relative organ weight study:** By physical precision balance and expressed in gram.

B. Hematological study:

1. Haemoglobin percentage (Hb %) in blood: By Sahli's acid haematin method.
2. Total Count of Red Blood Cell (TC- RBC): By new improved Neubauer counting chamber method.
3. Total Count of White Blood Cell (TC- WBC): By new improved Neubauer counting chamber method.

C. Serum biochemical analysis:

1. Follin-Phenol method was used for the determination of serum protein as described by Lowry *et al.* (1951).
2. The Dinitro Salicylic Acid method was used for the determination of serum glucose as described by Miller (1959).
3. The Ferric Chloride and Sulphuric Acid method was used for the determination of serum cholesterol as described by Henly (1957).
4. The Eriochrome Dye method was used for the determination of serum calcium as described by Preston Smith *et al.* (1966).

Statistical analysis

Standard error of mean (SEM) were calculated and p value of treated groups were also estimated by correlating them with control group using student 't' test described by Fisher and Yates, 1948.

RESULTS

Body weight & relative organ weight analysis:

1. **Body weight:** Aspirin (100 mg/kg b.wt.) did not affect body weight after 15 and 30 days of the treatments (table 1).
2. **Relative organ weight:** Aspirin (100 mg/kg b.wt.) did not affect relative organ weight after duration of the treatments (table 1).

Table (1): Effect of Aspirin on body weight (g) and relative organs weights (g) of rat, *Rattus rattus*.

Parameters	15 Days		30 Days	
	Control n=6	Treated n=6	Control n=6	Treated n=6
Body weight	125 ± 4.0	125.5 ± 5.0	127.5 ± 4.5	128.8 ± 5.9
Relative liver weight	3.87 ± 0.28	4.15 ± 0.31	3.873 ± 0.28	3.69 ± 0.18
Relative kidney weight	0.99 ± 0.11	0.88 ± 0.04	0.99 ± 0.11	0.76 ± 0.04***
Relative pancreas weight	0.36 ± 0.02	0.33 ± 0.02	0.36 ± 0.02	0.32 ± 0.02
Relative lung weight	0.86 ± 0.01	0.76 ± 0.12	0.86 ± 0.01	0.79 ± 0.12
Relative adrenal weight	0.02 ± 0.001	0.03 ± 0.001***	0.02 ± 0.001	0.03 ± 0.001***
Relative ovarian utricular weight	0.37 ± 0.04	0.40 ± 0.02	0.37 ± 0.04	0.38 ± 0.0631

± S.E.M. values of five animals, ***p< 0.001 (highly significant)

Hematological analysis:

1. **Haemoglobin percentage (Hb %):** Aspirin (100 mg/kg b.wt.) did not show much variations in Hb % (g/dl) (table 2).
2. **Total count RBC (TC-RBC):** Aspirin exposure (100 mg/kg b.wt.) did not significantly altered TC-RBC after 15 and 30 days (table 2).
3. **Total count WBC (TC-WBC):** Aspirin treatment (100 mg/kg b.wt.) significantly increase (<0.001) TC-WBC after 15 and 30 days (table 2).

Table (2): Effect of Aspirin on the haematological constituents of rat, *Rattus rattus*.

Parameters	Control (n=6)	Experimental groups	
		15 days n=6	30 days n=6
Haemoglobin (g/dl)	8.40 ± 0.02	8.42 ± 0.04	8.45 ± 0.04
TC - RBC (L/cumm)	6.075 ± 0.69	6.160 ± 0.63	6.465 ± 0.10
TC - WBC (T/cumm)	3187 ± 45.17	3457 ± 151.72***	3842 ± 13.38***

± S.E.M. values of five animals, ***p< 0.001 (highly significant)

Serum biochemical analysis:

1. **Serum glucose:** A daily dose of aspirin (100 mg/kg b.wt.) upto 15 and 30 days, significantly (<0.001) lowered serum glucose levels in comparison to the control group (table 3).
2. **Serum proteins:** Animals received a daily dose of aspirin (100 mg/kg b.wt.) through orally upto 15 and 30 days, did not altered significantly serum protein level as compared to the control group (table 3).
3. **Serum cholesterol:** Daily dose of aspirin (100 mg/kg b.wt.) received by the animals upto 15 and 30 days, lowered insignificantly serum cholesterol level in comparison to control group (table 3).
4. **Serum calcium:** No any much variations were noticed in serum calcium level of female rat, *Rattus rattus* after the daily dose of aspirin (100 mg/kg b.wt.) upto 15 and 30 days in comparison to control group (table 3).

Table (3): Effect of Aspirin on some of the serum bio-chemical constituents of rat, *Rattus rattus*.

Parameters	Control (n=6)	Experimental groups	
		15 days n=6	30 days n=6
Protein (mg/dl)	17.16 ± 0.29	11.85 ± 6.25	14.59 ± 4.25
Glucose (mg/dl)	131.94 ± 14.69	100.83 ± 15.10***	95.83 ± 16.10***
Cholesterol (mg/dl)	160 ± 10. 32	155 ± 8. 246	146 ± 5.63
Calcium (mg/dl)	12.03 ± 1.62	11.03 ± 0.63	11.03 ± 0.28

± S.E.M. values of five animals, ***p< 0.001 (highly significant)

DISCUSSION

Data of the present study indicate that, body weight and relative organs weights of rats (n=6) were not significantly affected by oral administration of aspirin (100mg/Kg b.w.) (table 1). This study also supported by other workers that oral administration of aspirin (0.05% w/v) for 30 days do not affect body weight in rats (Ebuehi, *et al*., 2007). On the other hand, in Vitamin E-Deficient Rats supplementation with aspirin resulted in an increase in their body weight (Machlin, *et al*., 1980).

The author suggested that, aspirin treated guinea pigs increase the production of blood eosinophils, basophils and absence of the changes in neutrophils (Hirai, *et al*., 2001). In our study the administration of aspirin (100mg/Kg b.w.) for 15 and 30 days do not altered TC RBC and Hb % significantly, while , ASA significantly (<0.001) increased TC WBC in 15 and 30 days treated rats (table 2). According to Otimenyin, *et al.* (2009) aspirin (260 mg/Kg b.w.) had showed no effect on packed cell volume, Hb %, RBC and WBC. On the other way, in ascites syndrome, hematocrit, RBC and Hb % appeared to decrease with increases in aspirin (from 0.05% to 0.20% w/v) level (Balog, *et al*., 2000).

The increase in TC WBC is due to, aspirin may inhibits PG synthesis through cyclooxygenase enzyme and enhances hematopoisis, because of PGE2 increases intracellular cyclic AMP levels in target cells and inhibit hematopoietic cells proliferation and maturation (Daud, *et al*., 2003). In addition to, cyclic AMP and PGE2 block neutrophils recruitment and aspirin enhanced by two fold neutrophils recruitment (Chignard, *et al*., 1996). It has also suggested that, small doses of aspirin do not change in the production of blood neutrophils, monocytes and lymphocytes probably because of, responsiveness of different blood cells by the products of arachidonate metabolism (Turja, *et al*., 2010).

Apart from this, the administration of aspirin for 15 and 30 days on rats significantly reduced the glucose level (table 3). As it is known that, ASA inhibit glycogenesis (resultant depletion of muscle and liver glycogen) and gluconeogenesis (due to inhibition of aminotransferase enzymes used for cytosolic malate and aspartate formation from mitochondrial pyruvate carboxylation, where they are used for gluconeogenesis) and uncoupling of oxidative phosphorylation (due to stimulation of oxygen consumption) resulted in a decrease in blood glucose levels (Myron, *et al*., 1971). Shaft, *et al.*, 1988 also showed that, the animals were given aspirin (30 mg/kg body weight) along with the daily dose of chlorpropamide causes more significant reduction of blood sugar level. As well as, oral administration of aspirin may reduce the level of corticosterone and T_3 hormone that consequently may affect glucose metabolism (Mohammed, *et al*., 2010).

The present study also showed that, aspirin (100 mg/kg b.w) given to rats did not induced any significant changes in serum proteins, cholesterol and calcium levels in albino rats (table 3). Balong, *et al*., 2000; Ibrahim and Gamal, 2003; Sherifa, 2006 have reported that asprin did not affect the level of protein, cholesterol, triglycerides and Calcium in rats. They showed that, aspirin and the salicylates are generally considered to be as a safe drug.

All these results suggested that, aspirin (acetylsalicylic acid) is a safe drug at low doses; apart from this it has some side effects also when administered at high doses. At high doses it impairs serum glucose level and TC- WBC by modulating of certain enzymes metabolism in albino rats.

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