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Research Article

ASPIRIN INDUCED CHANGES IN SERUM ACP, ALP, GOT, GPT, BILIRUBIN AND CREATININE IN CORELATION WITH HISTOPATHOLOGICAL CHANGES IN LIVER AND KIDNEY OF FEMALE ALBINO RAT

NEHA JAIN, RENU SHRIVASTAVA, ARUN K RAGHUWANSHI AND VINOY K SHRIVASTAVA*

Laboratory of Endocrinology, Department of Biosciences, Barkatullah University, Bhopal 462026 Madhya Pradesh, India.
Email: vinoyks2001@yahoo.com

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ABSTRACT

The aim of present investigation was to study the effect of oral administration of aspirin drug *i.e.* acetylsalicylic acid (ASA) on female albino rat, *Rattus norvegicus.* The animals (n=24) were allocated into 2 groups as control (n=12) and treated (n=12). The treated rats were administered orally (through gavage) a dose of aspirin 100 mg/ kg body weight for 15 days (n=6) and 30 days (n=6). The serum biochemical estimations such as ACP, ALP, GOT, GPT, bilirubin and creatinine levels along with histopathological study of liver and kidney were done. In present investigation, aspirin significantly elevated serum ACP, ALP, GOT, GPT, creatinine and bilirubin levels in both durations. In connection to this, it induced significant histopathological variations in liver and kidney after 15 and 30 days. All these results suggest that the high doses of ASA induced histopathological changes in liver and kidney directly or through modulation, certain biochemical and enzymological activities. These effects are dose and duration dependent.

Keywords: Aspirin (acetylsalicylic acid, ASA), Biochemical estimations, Histopathological study, *Rattus norvegicus.*

INTRODUCTION

Aspirin (acetylsalicylic acid, ASA) has been used as one of the most famous, cheap, easily available and widely used Non Steroidal Anti Inflammatory Drug (NSAID). Aspirin is used in versatile purpose such as, anti-inflammatory (in joint diseases), anti-platelets (in cardiovascular disease), analgesic and antipyretic[1]. Salicylic acid is metabolized via conjugation in the liver to form salicyluric acid and several other metabolites. It is also well known that, aspirin is rapidly absorbed from the stomach and small intestine, primarily by passive diffusion across the gastrointestinal (GI) tract which rapidly hydrolyzed to salicylic acid by esterase in the GI mucosa and blood plasma. It dispersed throughout the body after ingestion, with the highest concentrations found in the blood plasma, liver, renal cortex, heart and lungs[2].

Aspirin is a safe drug at low doses but also it has life-threatening side effects when administered at high doses. Long-term therapeutic administration of aspirin is associated with nephrotoxicity, hepatotoxicity, gastrointestinal ulcerations, and even renal cell cancer due to its adverse effects on multiple organ systems[3,4]. In connection to this also reported that, it can cause adverse affects in pregnancy[5]. In-vitro and in-vivo studies showed that aspirin at high doses caused death of the blood vessel tissues[6]. The inhibitory activity of aspirin also found on the endocrine hormones *viz.* ACTH, endorphin, cortisol, prolactin and growth hormone via possible stimulatory role of prostaglandin. Also, overdose of aspirin stimulate corticosteroid secretion by the adrenal cortex[4].

Aim of the work

The aim of the present study is to observe the side effects of the anti-inflammatory drug aspirin (100 mg/ kg body weight) on liver and kidneys of young albino female rats, *Rattus norvegicus,* by observing histopathological studies in liver and kidney and by estimating enzymological *i.e.* ACP (Acid Phosphatase), ALP (Alkaline Phosphatase), GOT (Glutamate Oxaloacetate Transaminase), GPT (Glutamate Pyruvate Transaminase) and certain biochemical *i.e.* bilirubin and creatinine levels in serum after 15 and 30 days of the treatments.

MATERIALS AND METHODS

Material

Aspirin (acetylsalicylic acid) tablets (75 mg/tablet) as anti-inflammatory drug was purchased from the market, trade name Ecospirin-75 (manufactured by USV LTD, B.S.D. Marg, Govandi, Mumbai-400088).

Experimental animals

The above study was carried out on 24 adult female albino rats (body weight ranging between 150-200 gm) were kept into polypropylene cages and acclimatized to laboratory condition at 23-25ºC temperature with 14 hours light and 10 hours dark cycle at least for 7 days prior to initiating to the experiment. The study was conducted as per guidelines of Committee for the purpose of control and supervision on experiments on animals (CPCSEA), Chennai and maintained in the Laboratory of Bioscience, Barkatullah University. The animals were fed with standard rat feed and water *ad libitum*. A dose of aspirin (100mg/kg b. wt.) was daily administered orally, through gavage (administration of food or drugs by force, especially to an animal) for 15 and 30 days. The 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 given aspirin orally (100mg/kg b. wt.) through gavage for 15 days.

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

After above treatments, the animals were weighed, sacrificed on 16th and 31st day, blood samples were collected through cardiac puncture, serum were separated for enzymological and biochemical estimations and organs like liver and kidney were preserved in Bouin's fluid for histopathological observations. Therefore, following parameters were done by appropriate methods.

Parameters

Serological analysis

1. King and Kings method was used for the determination of serum Acid Phosphatase (ACP) and serum Alkaline Phosphatase (ALP)[7].
2. Reitman and Frankel method was used for the determination of serum Glutamate Oxalate Transaminase (GOT) and serum Glutamate Pyruvate Transaminase (GPT)[8].
3. Malloy and Evelyn method was used for the determination of serum Bilirubin[9].

4. Alkaline picrate method was used for the determination of serum Creatinine[10].

Histopathological study

For histological study liver and kidneys were dissected out quickly, cleaned, dried with blotting paper and fixed in Bouin's fluid. The classical paraffin sectioning (7µ thick) were cut, stained with Haematoxylin and Eosin staining and observed under light microscopy for histopathological changes[11].

Statistical analysis

The 'p' value of treated v/s control were calculated by adopting Student't'-test[12].

RESULT AND DISCUSSION

Aspirin develops hepatotoxicity *i.e.* lethal hepatocellular injury and hepatic massive micro-steatosis via mitochondrial dysfunction and lipid peroxidation mechanism resulting marked fall in intracellular ATP and disrupt free fatty acid accumulation in liver [13,14]. Aspirin also develops nephrotoxicity *i.e.* vacuolar degeneration of tubules and gradually elevates membrane associated phosphatase (ACP and ALP) and transaminase (GOT and GPT) enzymes of tissues, these enzymes maintain the amino acid homeostasis[15,16,17]. It also increases serum bilirubin and creatinine content in aspirin treated patients due to hepatic and renal toxicity[15]. Due to, aspirin administration mainly induced vasoconstriction and smooth muscle atrophy in liver and kidney via inhibition of the synthesis of different prostaglandin viz. PGE2, PGD2, PGF2 and PGI2 which are potent vasodilators[1,18].

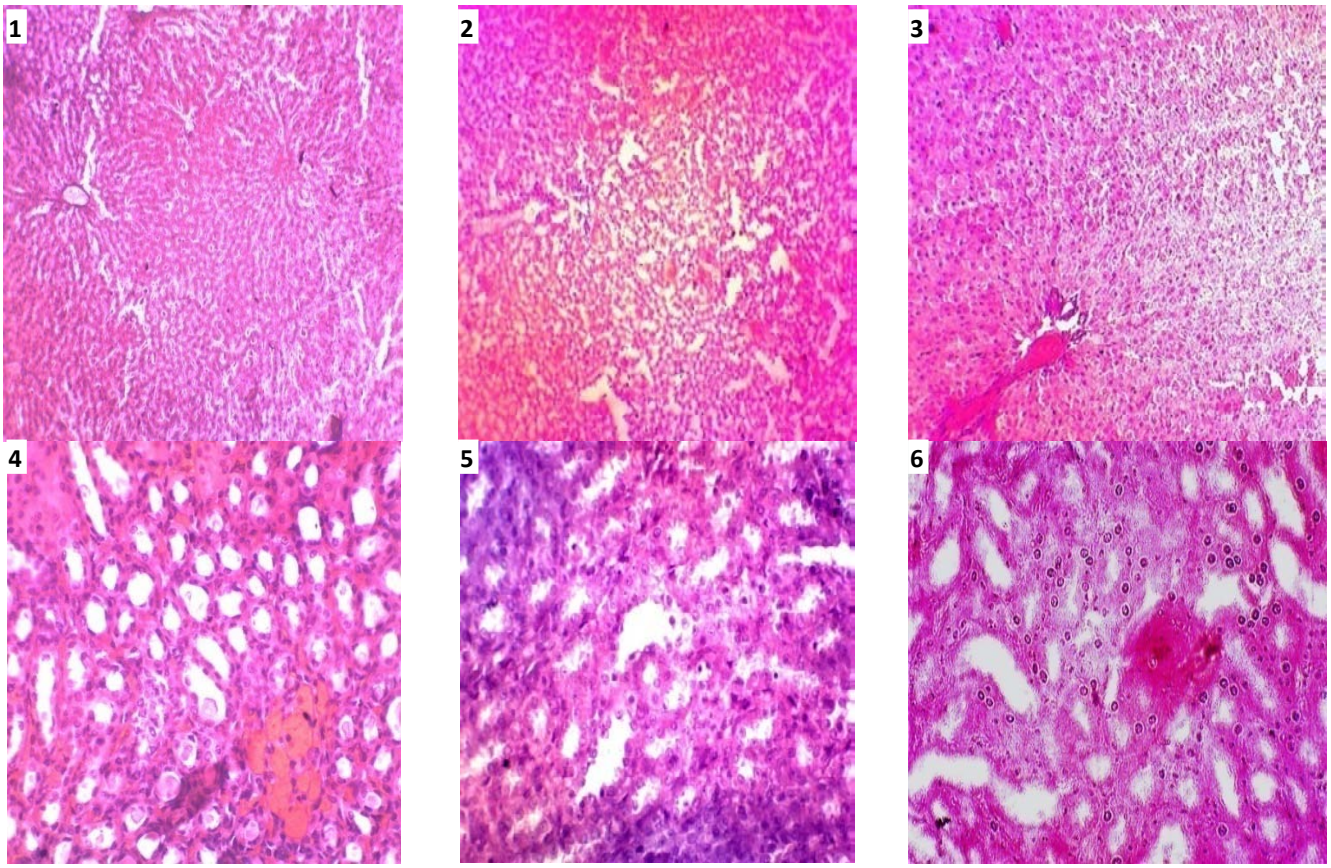
In present study, aspirin (100mg/Kg b.wt.) significantly elevated ACP, ALP, GOT, GPT, creatinine and bilirubin content in serum upto 15 and 30 days treatment (Table 1).

And, the administration of aspirin caused significant histo-pathological changes in liver and kidney (Fig. 1-6). Aspirin treated liver of rat showed degenerative and pycknotic changes in the nuclei with less cytoplasmic materials having dense particles in the hepatocytes. As well as, vacuolization, clear dilations were also found in the sinusoids, most of the hepatocytes become hypertrophied in condition with small and degenerating nuclei and lobules have become fused in later part of the experiment (Fig. 2, 3). In connection to this, aspirin treated kidney of rat showed atrophic changes in the proximal and distal convoluted tubules in the cortex, the inflammatory changes were noticed in medulla and damaged tubular epithelial cells by ischemia due to vasoconstriction of renal arterioles (Fig. 5, 6). Renal vascular tone was determined by autonomous intrinsic activity of the renal arterioles and continuous production of prostaglandin which is inhibited by this drug and thus caused unopposed constriction of arterioles resulting in ischemia of tubules and epithelial cell death[19]. Other workers also suggested that aspirin caused vacuolar degeneration of proximal tubules, focal tubular atrophy and significantly decreased proximal tubules per unit area, resulting renal failure [20,21].

Table 1: Effect of aspirin on serum bio-chemical constituents of rat, *Rattus norvegicus*.

Parameters (mg/dl)	Control	15 days treated group	30 days treated group
ACP	9.23 ± 0.44	13.44 ± 3.58***	14.59 ± 0.28***
ALP	10.37 ± 0.63	12.56 ± 1.01***	15.58 ± 0.60***
GOT	7.33 ± 0.63	16.58 ± 0.56***	20.16 ± 0.63***
GPT	6.55 ± 1.33	14.55 ± 1.33***	15.55 ± 1.33***
Creatinine	0.55 ± 0.44	2.18 ± 0.33***	4.33 ± 0.63***
Bilirubin	0.11 ± 0.06	0.24 ± 0.06***	0.23 ± 0.06***

Mean ± SD, n = 6, ***p< 0.001(Highly significant)



Explanation of figures of Liver and Kidney

Fig 1. Section of liver of control *Rattus norvegicus* showing normal features of hepatocytes having prominent and spherical nuclei with defined cytoplasmic materials (H & E 400X).

Fig 2. Aspirin treated (100 mg/kg) liver of *Rattus norvegicus* upto 15 days showing hypertrophied and degenerative hepatocytes with pycknotic changes in the nuclei having less cytoplasmic materials (H & E 400X).

Fig 3. Aspirin treated (100 mg/kg) liver of *Rattus norvegicus* upto 30 days showing vacuolization, lobules have become fused, clear dilations found in sinusoids, most of the hepatocytes become hypertrophied in condition with small and degenerating nuclei (H & E 400X).

Fig 4. Cortical region of kidney of control *Rattus norvegicus* showing normal interstitial connective tissue, peri-tubular capillaries, proximal convoluted tubules (PCT) (having prominent brush border, wide lumen) and distal convoluted tubules (DCT) (having narrow lumen) which have prominent and spherical nuclei (H & E 400X).

Fig 5. Aspirin treated (100 mg/kg) cortical region of kidney of *Rattus norvegicus* upto 15 days showing normal appearance of PCT and DCT but vacuolar degeneration found (H & E 400X).

Fig 6. Aspirin treated (100 mg/kg) cortical region of kidney of *Rattus norvegicus* upto 30 days showing PCT lumen cells which clogged with tall microvilli, damage in the tubular epithelial cells, vacuolar degeneration and marked tubular atrophy with interstitial fibrosis (H & E 400X).

CONCLUSION

Finally, it may be concluded that aspirin at the dose of 100mg/kg b.wt. caused significantly histo-morphological variations in liver and kidney via increasing ACP, ALP, GOT, GPT, creatinine and bilirubin levels in serum of young albino female rats, *Rattus norvegicus*. If it is taken longer duration by patients; they may suffer from renal and hepatic problems.

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