ORIGINAL ARTICLE

Acetylsalicylic Acid Effect over Testicular Weight and Seminiferous Tubules Diameter

MUHAMMAD KAMRAN AMEER[1], SABAHAT GULL[2], FAIZA MEHBOOB[3], *WAJIH-UR-REHMAN[4], MUHAMMAD WAQAS[5], ASMA SAEED[6]*

ABSTRACT

Aim: To observe the change in testicular weight and seminiferous tubules diameter in male albino mice after ASA administration.
Place and duration of study: One-year Research work at University of Health Sciences Lahore
Methods: Twenty seven male adult albino mice were randomly divided into three groups, each consisting of nine animals. The control Group A was given 10 ml/kg of distilled water orally. The experimental Groups B and C were orally given Aspirin, once daily dose of 100 mg/kg and 25 mg/kg respectively. The drug administration continued for 30 days and after 24 hours of last dose all the animals were weighed, anesthetized and sacrificed. The testes from each mouse were removed, weighed and processed for H & E staining. The relative tissue body weight index was calculated with the body weight and the testes weight. Mean tubular diameter was calculated by taking the average diameter of thirty tubules from each mouse.
Results: The mean of paired testicular weight, RTWI and tubular diameter was significantly raised (P value ≤ 0.05) in groups B & C as compared to control group A.
Conclusion: ASA enhances the process of spermatogenesis.
Keywords: Mice, Spermatogenesis, ASA (Acetylsalicylic acid), RTWI (Relative tissue weight index).

INTRODUCTION

Primary male infertility is defined as failure to father a child after 12 months of regular unprotected intercourse with the same partner[1]. Eight percent of the male population of Pakistan is suffering from the issue of infertility[2]. Worldwide the situation is becoming alarming due to exposure to lot of environmental hazardous agents and lifestyle changes[3].

Spermatogenesis, a vital function of the male gonads, consist of three distinct phases; Proliferation, Meiosis and Differentiation[4,5]. Male gonads may be affected in genetic disorders like Klinefelter's syndrome, infective orchitis, radiations, drugs, torsion and varicocele leading to infertility[6].

Acetylsalicylic acid (ASA) is a non-steroidal anti-inflammatory agent along with powerful anti-platelet action[7]. Since decades it had been used as an antipyretic and analgesic agent[8]. In the modern era it is an essential drug for the prevention of ischemic heart diseases[7]. It adversely affects the gastric mucosa leading to ulcer, perforation and bleeding[9]. Being irreversible inhibitor of cyclooxygenase I & II Aspirin inhibits the production of prostaglandins (PGE_2, PGF_2, PGH_2 and PGI_2) prostacyclin (PGI_2) and thromboxane (TXA_2) until the cyclooxygenase concentration is restored by the nuclear DNA[10].

ASA influence over gonadal functions has long been investigated but the established facts are insignificant and controversial. Kumar and Chinoy[11] verified that ASA treatment markedly alters the metabolism and decreases the weight of testis. Tanyildizi and Bozkurt[12] observed that sperm motility increased after treatment with ASA and metimazol in rams.

The present study is designed to investigate the effect over testicular weight and seminiferous tubules diameter after ASA administration.

MATERIAL AND METHODS

Twenty seven male Albino mice of age almost 7 weeks and weight 25 to 35 gram were gathered from veterinary Research Institute Lahore. All the animals are examined and kept in animal research laboratory of University of Health Sciences, Lahore. Under the control environment and were fed on normal chow and water ad libitum. The albino mice were randomly divided into three groups of nine. Aspirin was commercially procured in the powdered form. 1 gram of Aspirin was dissolved in 100 ml of distilled water[13]. The low and the high dose of Aspirin was calculated by human equivalent dose (HED) method[14,15]. The animals were weighed daily for determining the required dose of aspirin. The drug was gently passed into the esophagus using an oral gavage.

Group A that served as a control was given 10 ml/kg of distilled water orally for 30 days.

Group B was given Aspirin at a high dose of 100 mg/kg dissolved in 10ml distilled water orally for a period of 30 days. Group C was given Aspirin at a low dose of 25 mg/kg dissolved in 2.5 ml distilled water orally for a period of 30 days.

On 31st day of experiment all the animals were weighed, anesthetized and sacrificed. The Abdominal cavity was explored with a vertical midline incision. The testes were pulled into the abdominal cavity and removed along with the epididymis, vas deferens and Spermatic blood vessels.

[1,3]Assistant Prof. Anatomy, Multan Medical & Dental College. Multan.
[2]Assistant Professor of Anatomy, Quaid e Azam Medical College. Bahawalpur.
[4]Assistant Professor of Medicine, Quaid e Azam Medical College. Bahawalpur.
[5]SR Urology, Quaid e Azam Medical College. Bahawalpur.
[6]Associate Professor of Pharmacology, Multan Medical & Dental College. Multan.
Correspondence to Dr. Muhammad Kamran Ameer,
Email: noshee05@hotmail.com, kamrananatomy@hotmail.com

After removing the epididymis, the Weight of the paired testes was measured in grams by using the digital balance. The relative tissue body weight index (RTW1) was calculated by the following formula

RTWI = Paired weight of testes (g) X 100 / body weight (g)

The testes were transversely sectioned slightly away from the midline and immersed in the bouin's fixative for 48 hours. The tissue pieces were then washed and processed in Automatic tissue processor. Afterwards the tissue was placed in the paraffin block and freeze. Four micrometer thick sections were obtained by using the rotatory microtome. The prepared slides from the tissue blocks were stained with H & E stain.

For Calculating the Mean tubular diameter, ten uniform and rounded seminiferous tubules were examined from each of three slides prepared from the specimen of each animal. A total of three ninety circular tubules were used for Calculating the mean of a group.

Statistical analysis: Data was analyzed by SPSS version 18. Mean and standard deviation were calculated for weight, RTWI & tubular diameter. One-way ANOVA followed by Post Hoc Tukey test were applied to compare the variables

RESULTS

There was statistically significant decline (P value ≤ 0.05) in the mean body weight of group B and group C as compared to control group A. The mean of paired testicular weight, RTWI and tubular diameter was significantly raised (P value ≤ 0.05) in the experimental group B & C as compared to control group A

Parameter	Group A	Group B	group C
Mean Body Weight (gm)	35.61±3.6	29.98±2.4	29.54±5.5
Mean Weight of Paired Testes (gm)	0.119±0.015	0.156±0.02	0.149±0.024
Mean RTWI	0.34±0.050	0.52±0.094	0.46±0.122
Mean Tubular Diameter	169.4±14.1	188.0±20.1	187.96±17.9

Photomicrographs of histological section of testes from Group A (Fig. 1) Group B (Fig. 2) and Group C (Fig. 3) showing the measurements of seminiferous tubules' diameter. H&E stain X100.

Fig. 1

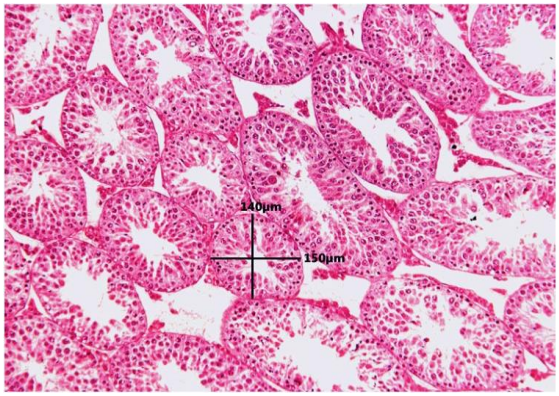


Fig. 2:

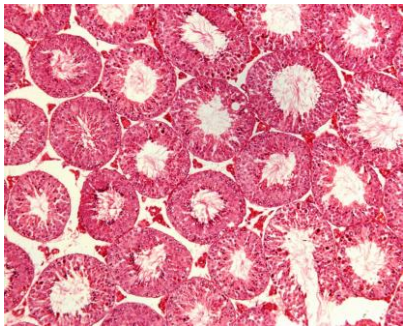
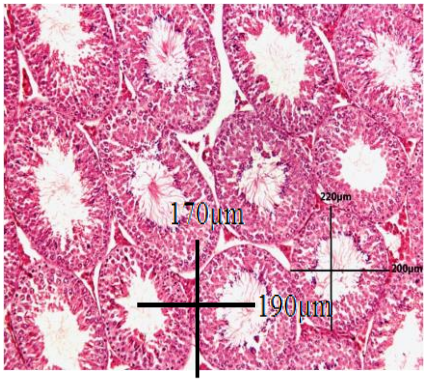


Fig. 3:



DISCUSSION

The drug significantly increased the mean weight of the testes and tubular diameter. There was even gain of weight in the control groups during the period of experiment while in the groups B and C there was decline in the body weight during first fifteen days then it raised. The initial loss of body weight in ASA treated groups (B & C) is possibly due to gastric intolerance, which disturbs the appetite[9]. Adverse effect on the health of animals was obvious by their sluggish physical activity. Chaloob et al[16] and Millis et al[17] had the same observations of weight loss when they treated their research animal with ASA.

The increase in paired weight of testis in ASA treated groups B & C was statistically significant as compared to the control. The mean value of paired weight was higher in group C receiving a higher amount of ASA, but the difference was insignificant. The relative tissue body weight index in ASA treated groups B & C increased significantly against the control.

This upsurge of testicular weight may be credited to the increase in number of germ cells and diameter of seminiferous tubules. The growth of testicular weight is attributed to the androgenic stimulus as evident by increased tubular diameter[18]. Ratnasooriya and Lionel[19] locally injected meclofenac (non-steroidal anti-inflammatory agent) filled rods in rats for a period of 60 days and noticed increase in weight of testis, epididymis and vas deference. The findings, that Didolkar[14] et al mentioned contradict with our results as they reported decline in testicular weight after ASA administration to rats.

The mean tubular diameter of seminiferous tubules of ASA treated groups B & C was significantly higher than the control group. In contrast, Chaloob et al[16] described the ASA decreases tubular diameter in albino mice. Stutz et al[20] also concluded that aspirin like drugs have no deleterious effect on sperm quality. This increase in tubular diameter indicates that ASA promotes spermatogenesis as Bustos-Obregon[21] described that this hypertrophy is associated with an adaptation process to compensate the increased spermatic activity.

CONCLUSION

After the analysis of observed data i.e., increase in testicular weight, relative tissue weight index and the mean tubular diameter it is suggested that ASA treatment in mice promotes spermatogenesis.

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