

COMPARATIVE TOXICITY OF FIVE COMMONLY USED ANALGESICS ON RAT SPERM COUNT AND SPERM MORPHOLOGY.

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ABSTRACT

Five commonly used analgesics (Aspirin, Cafenol, Panadol, Pentax and Daga⁺) with "Aspirin+Paracetamol+Caffeine" combinations were administered orally to albino rats in drinking water for 90 days to determine their toxic effects on sperm anogenesis. Maximum recommended daily dosage of 67.2 mg/kg for Aspirin, 118.9 mg/kg for Cafenol, 79.5 mg/kg for Panadol, 138.1 mg/kg for Pentax and 152.2 mg/kg for Daga⁺ were administered. The sperm cells were harvested by one epididymis from each rat being weighed and minced with fine scissors into 1mg aliquots in physiological saline and after vigorous pipetting, the suspension was separated from tissue fragments by filtering it through a 80µm stainless mesh and the yield of sperm per milligram (mg) of epididymis was determined using the improved Neubauer haemocytometer. Significant reduction ($p < 0.001$) in Mean Sperm Count of the treated rats was observed in the following order: Pentax ($44,850 \pm 49$) > Cafenol ($35,480 \pm 31$) > Aspirin ($32,950 \pm 38$) > Panadol ($23,700 \pm 27$) > Daga⁺ ($3,040 \pm 25$) as against $46,250 \pm 72$ /mg of epididymis in control. An inverse relationship of Mean Sperm Count with total percentage of sperm head abnormalities was observed as there were significant increases in sperm head abnormalities for treated rats in the order: Pentax ($p < 0.05$), Cafenol, Aspirin, Panadol and Daga⁺ ($p < 0.001$) of 4.0, 7.5, 8.0, 10.5 and 13.5% respectively as against 1.5% recorded in control. The five tested analgesics show significant effects on Mean Sperm Count and total percentage of sperm head abnormalities, therefore confirming their toxicity on albino rats as a model.

KEYWORDS: Toxicity, Sperm Count, Sperm Head Abnormalities, Analgesics

INTRODUCTION

In recent years, there has been an increasing awareness and realisation of the genotoxic potentials of a wide variety of drugs, food additives, environmental pollutants (Jones and Bodmer, 1974; Taylor, 1980) and other socially acceptable compounds, such as habitual ingestion of aspirin and caffeine which are capable of inducing structural chromosomal aberrations in somatic cells in vivo (Neel and Bloom, 1973). This awareness follows the recent development of appropriate, sensitive and practical methods for detecting and estimating the toxic effects of these substances and their impact on environmental health.

Commonly used (non-prescription, over-the-counter) analgesics normally come in combinations of aspirin or paracetamol or both. There are often combined with caffeine to augment their antipyretic and analgesic effects (Meyers et al., 1979). These analgesics are ingested for many reasons including: general pains, headaches, fevers, cold, flu, rheumatoid arthritis, circulation problems (Faulkner et al., 1998) and in cases of dependent-addiction (Ross et al., 1989).

DNA synthesis in the testis, spleen, thymus, stomach, small intestine and bone marrow was reported to have been inhibited by 70-90% at 1hour following

an oral dose of 1g/kg of Paracetamol in rats (Lister and McLean, 1997). Increases in incidence of abnormal sperm has been reported in albino rats treated with formaldehyde (Odeigah, 1997) and also high temperatures, extreme nutritional deficiencies and some diseases in a wide ranged of species including mice and man (Obe and Ristow, 1979). A mean frequency of 1.2% abnormalities was reported for control groups (Van Thiel et al., 1975).

Anti-inflammatory analgesics and certain steroids inhibit the production prostaglandins (Hole, 1993); and inhibition of prostaglandins interferes with spermatogenesis (Bruce and Heddle, 1979; Wyrobek and Bruce, 1979; Letz, 1990; Hole, 1993; Lister and McLean, 1997) and could result in functional and structural impairment of sperm cells (Wyrobek and Bruce, 1978; Letz, 1990).

In view of above finding, this study set out to explore further the toxic effects of five commonly used analgesics (Aspirin, Cafenol, Panadol, Pentax and Daga⁺) with "Aspirin+Paracetamol+Caffeine" (APC) combinations on Mean Sperm Count (MSC) and total percentage of Sperm head abnormalities (SHA) of albino rat as a model; using two short-term in vivo mutagenicity assays: Mean Sperm Count and Sperm head abnormality test (SHAT).

MATERIALS AND METHODS

(a) Animal husbandry: Isogenic strains of male albino rats (*Rattus norvegicus*) were obtained from the rat colony of the Biological Garden, University of Lagos, Akoka, Lagos for the study. Rats used for the test were 12 to 14 weeks old. All rats were housed in conventional cages and maintained on food (Rat pellets from Livestock Feeds Limited, Ikeja, Lagos) and water ad libitum. Forty-eight male rats of almost the same body weight were randomly assigned to groups of 8 male rats for each treatment and control. Daily water intake and body weight were measured during the acclimatization period of 4-8 weeks and during the study.

(b) Treatment: The following five commonly used analgesics were obtained from reputable pharmacies; their tablets were weighed and ground into powdery form:

(i) Aspirin from Vitalink (Weight: 336mg; Content: Aspirin 300mg),

(ii) Cafenol from Sterling Winthrop (Weight: 446mg; Content: Aspirin 375mg, Caffeine 25mg),

(iii) Panadol from Sterling Winthrop (Weight: 596mg; Content: Paracetamol 500 mg),

(iv) Pentax from Vitabiotics (Weight: 518mg; Content: Paracetamol 500mg, Caffeine 16mg) and

(v) Daga⁺ from Hoechst (Weight: 761mg; Content: Aspirin 225mg, Paracetamol 250mg, Caffeine 30mg).

The daily doses (D) were calculated taking the average human weight as 60 kg (Werner, 1982) and using the formula: $D = MRT$ (where, M = Maximum recommended daily human dosage in tablets, R = Ratio of weight of rat to average adult human weight, T = Weight of each tablet). The required maximum recommended daily dosage of 67.2 mg/kg for Aspirin, 118.9 mg/kg for Cafenol, 79.5 mg/kg for Panadol, 138.1 mg/kg for

Pentax and 152.2 mg/kg for Daga⁺ were dissolved in about 60% average daily water intake determined during period of acclimatization, this was to ensure that the daily doses were consumed; before adding more water.

(c) Mean Sperm Count (MSC): This was carried out according to the method of Topham (1980). The rats were sacrificed after 90 days of dosing, and the sperm count/mg of epididymis determined by haemocytometry as follows: one epididymis from each rat was weighed and minced with fine scissors into 1mg aliquots in physiological saline. After vigorous pipetting, the suspension was separated from tissue fragments by filtering it through a 80µm stainless mesh and the yield of sperm per milligram (mg) of epididymis was determined using the improved Neubauer haemocytometer. Differences between the means of the control and experimental groups were compared using the Student's (t) test.

(d) Sperm head abnormality test (SHAT): A fraction of each suspension was mixed with 1% eosin Y solution (10:1) for 30 minutes and air-dried smears were prepared on glass slides. The slides were coded, randomised and examined for percentage abnormalities in every 200 spermatozoa from each slide and one thousand spermatozoa from each of the 8 rats in each treatment group and control. Differences between the control and experimental groups were compared using the Chi-squared (χ^2) test.

RESULTS

Five haemocytometer counts were taken from each of the 8 rats in control and each treatment group. For the control, the mean sperm count/mg of epididymis for each of the 8 rats were 46,150; 46,180; 46,200; 46,240; 46,250; 46,300; 46,330 and 46,350. The

Table 1: Frequency Of Abnormal Sperm Heads

Treatment	Frequency of abnormal sperm head (%)						Total Frequency (%)
	Nubbed (Hook appear nubbed)	Long (Hook is elongated more than normal)	Short (Hook is shorter than normal)	Pin (No hook, pin-head shaped)	Dunce cap (Hook appears like Dunce cap)	Wrong angle (Hook is wrongly situated.)	
Control	1.0	0.5	0.0	0.0	0.0	0.0	1.5
Pentax	2.5	0.5	0.5	0.5	0.0	0.0	4.0 *
Cafenol	4.5	1.5	1.0	0.5	0.0	0.0	7.5 **
Aspirin	5.0	1.5	1.0	0.5	0.0	0.0	8.0 **
Panadol	5.5	2.0	1.0	1.0	1.0	0.5	10.5 **
Daga ⁺	7.5	2.0	1.5	1.0	1.0	0.5	13.5 **

* Significantly increased over control, $p < 0.05$, chi-squared (χ^2) test.

** Significantly increased over control, $p < 0.001$, chi-squared (χ^2) test.

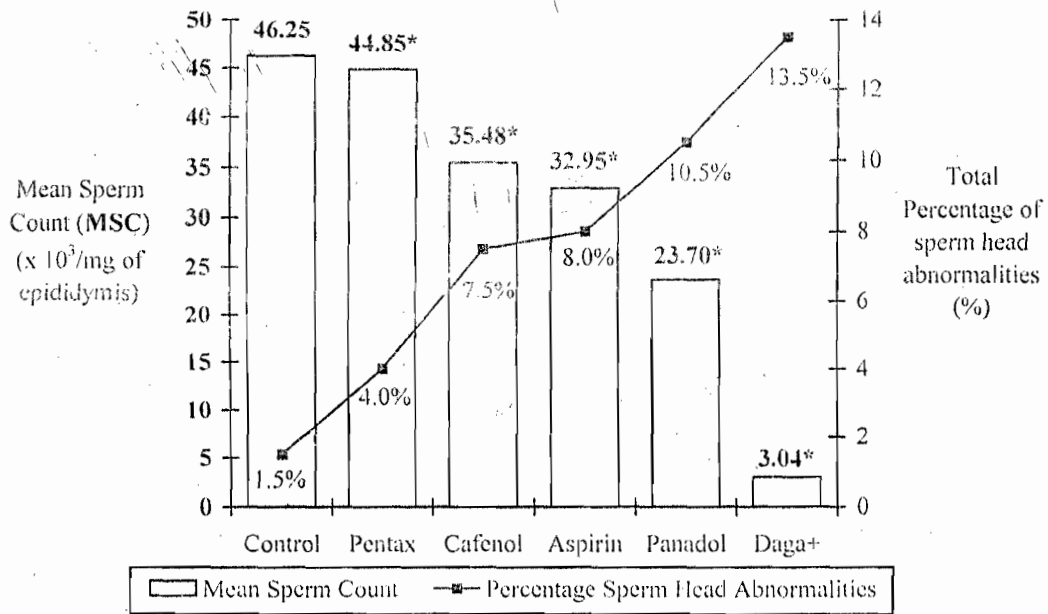


Figure 1: Relationship Between Mean Sperm Count And Sperm Head Abnormalities.

*Significantly decreased below control, $p < 0.001$, Student's (t) test.

computed mean of means was $46,250 \pm 72$. For rats treated with Pentax, their mean of means was $44,850 \pm 49$ while the individual means were 44,780; 44,800; 44,820; 44,840; 44,860; 44,880; 44,900 and 44,920. For Cafenol, their mean of means was $35,480 \pm 31$ and the individual means were 35,430; 35,450; 35,460; 35,480; 35,490; 35,500; 35,510 and 35,520. For Aspirin, their mean of means was $32,950 \pm 38$ while the individual means were 32,890; 32,920; 32,930; 32,940; 32,950; 32,980; 32,990 and 33,000. For Panadol, their mean of means was $23,700 \pm 27$ while the individual means were 23,660; 23,680; 23,690; 23,700; 23,710; 23,710; 23,720 and 23,730. For Daga+, their mean of means was $3,040 \pm 25$ while the individual means were 3,010; 3,020; 3,020; 3,030; 3,040; 3,050; 3,070 and 3,080. Six different types of sperm head abnormalities were observed. Of these, nubbed hook (hook appear nubbed) and long hook (hook elongated more than normal) were the most common as shown on Table 1. An inverse relationship of Mean Sperm Count (MSC) with total percentage sperm head abnormalities (SHA) was observed as there were significant increases in (SHA) for treated rats in the order: Pentax, ($p < 0.05$), Cafenol, Aspirin, Panadol and Daga+ ($p < 0.001$) of 4.0, 7.5, 8.0, 10.5 and 13.5% respectively as against 1.5% recorded in control as shown on Figure 1.

DISCUSSION

Increases in the incidence of abnormal sperm have been reported after treatment of male albino rats with formaldehyde (Odeigah, 1997). It has been reported that high temperatures, extreme nutritional deficiencies and some diseases can cause sperm abnormalities in a wide range of species including mice and man (Obe and Ristow, 1979). Male Wister rats fed on a diet containing 36% of total calories as ethanol for 41 days caused similar sperm abnormalities as those

74 reported in other mammalian species, and the mean frequency in control groups was 1.2%, with the range 1.0-1.6% (Van Thiel et al., 1975). The frequency of the control group here is also within this range and frequencies of the treated groups were significantly above the control ($p < 0.05$) for Pentax and ($p < 0.001$) for Aspirin, Panadol and Daga+ respectively, which agrees with Topham (1980). Various anti-inflammatory analgesics and certain steroids inhibit the production of prostaglandins (Hole, 1993), and inhibition of prostaglandins is known to interfere with spermatogenesis. Hence the reductions in sperm count. The morphological abnormalities might have been caused by alterations (deletions, point mutation or a combination of both) in testicular DNA that in turn disrupts the process of differentiation of spermatozoa (Bruce and Heddle, 1979; Lister and McLean, 1997); exposure to chemicals that could produce pituitary-hypothalamic or sex hormonal effects which in turn could affect spermatogenesis (Wyrobek and Bruce, 1978; Letz, 1990; Hole, 1993), and exposure of the seminal fluid to chemicals, resulting in functional or structural impairment of sperm cells (Wyrobek and Bruce, 1978; Letz, 1990). The tested analgesics showed significant effects on sperm head abnormalities with the following trend: Pentax < Cafenol < Aspirin < Panadol < Daga+. This is due to the active ingredient(s) found in the analgesics. The presence of Caffeine with "Aspirin" or "Paracetamol" alone reduced the toxicity of the combinations as in Cafenol (Aspirin + Caffeine) and Pentax (Paracetamol + Caffeine) when compared to that of Aspirin (Aspirin alone) and Panadol (Paracetamol alone) respectively, while the presence of Caffeine with "Aspirin + Paracetamol" increases the toxicity of the combinations by about 2-fold as in Daga+ (Aspirin + Paracetamol + Caffeine). This conforms to the findings of Jones and Bodmer (1974); Patierno et al. (1989), which shows that

the presence of caffeine in "Aspirin + Paracetamol + Caffeine" combinations increase the toxicity of the combinations by about 2-fold over "Aspirin" and "Paracetamol" alone.

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