



Original Article

Evaluation of the Changes in Sperm Morphology, Sperm Count and Gonadotrophic Ratio of Swiss Albino Male Mice Fed Continuously With Microwave Exposed Food

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ABSTRACT

With the technological advancements the dependence of man on electronic gadgets has increased many folds. The electronic gadgets have entered in man life from bedroom to kitchen. One such invention is microwave which has reduced the cooking time significantly. The harmful effects of microwave came into light after the Swiss and German research (Humbolt-Universitat zu Berlin 1942-1943, the Institute of Radio Technology at Kinsk, 1957). In the present study the experimental animals: Swiss Albino male mice, were divided into 3 groups Control, Sham and Experimental. The sham group was given the normal food in low quantity whereas control was given normal food in sufficient amount and experimental group were provided microwave exposed food pellets at 320°C for 10 minutes. Mice were fed with the fixed quantity for 2 weeks, 3 weeks, 4 weeks and 4 weeks recovery was fed with microwave exposed food for 4 week after that they were fed on normal feed for 4 weeks (Recovery group). The body weight and gonadotropic ratio showed marked fluctuations whereas the sperm count from 2 weeks to 4 weeks showed significant reduction. After the replacement of treated food with normal food, recovery was observed. The gonadotropic ratio declined significantly. The level of deformities, like coiled tail, oval head, cytoplasmic droplets, and bifurcated tails, continuously increased with increasing duration of feeding of microwave exposed food. Keywords: Microwave cooked food, sperm count, sperm morphology, gonadotropic ratio.

INTRODUCTION

With increasing urbanization and scientific development our need for electronic gadgets has increased a lot. Our dependence on gadgets leads to more inventions and it has now become an integral part of everybody's life. From home to offices electronic equipments are now part of our day to day activities as it eases much of our loads. Though, it has made many things easier but it has a great impact on our lifestyle patterns. One such invention is microwave technology. Microwave technology has been widely used in national defense, industrial and agricultural production, transportation, communications, information industry, medical and scientific research fields, to the people's work and life. A microwave oven is a kitchen appliance that heats food by dielectric heating. This is accomplished by using microwave radiation to heat polarized molecules within the food. Water molecules present in the food are bipolar, they absorb microwaves and cause heating of food items. Microwave exposure caused a higher degree of protein unfolding than usual thermal stress at the same temperature [1]. Additionally, microwaving creates new compounds that are not found in humans or in nature called radiolytic compounds [2]. There are reports that microwave radiations altered amino acids in milk and food to toxins for both the nervous system and the kidneys [3]. Yuan [4] found that microwaving food produced more acrylamide than boiling or frying (at 180°C), and that 750 Watt ovens produced more acrylamide (cancer causing) than 500 Watt ovens. Microwaving may even result in the development of new, hitherto unknown substances. The microwaves-induced reversal of the polarity, causes the cells in the nutrients to become destructively polarized, possibly allowing for the creation of free radicals [5]. All free radicals have a strong tendency to cause reactions. They can interact with enzymes thus causing a disruption of biological processes. In addition, through induction the food itself becomes a carrier and secondary source of technically generated radiation. William Kopp [6] observed that microwave exposure adversely affect the essential nutritional components of a food, like vitamins,

minerals, nucleoproteins and also lowers the metabolic activity of alkaloids, glucosides, galactosides and nitrilosides besides structural disintegration in all foods. As a result microwave ovens were banned in Russia in 1976, but ban was lifted later.

In the study conducted by Cornell University in 1977 some sugar was irradiated and it was fed to rats. The type of cell damage shown on post mortem was the same as if the rats themselves had been irradiated.

The present work is designed to study the changes in sperm parameters of mice provided with the microwave exposed food for different duration. Recoveries in the parameters were also observed after withdrawing the exposed food.

MATERIALS AND METHODS

Animal and treatment: Sexually mature male mice (*Mus musculus*) weighing between 25 to 30 g were randomly selected. They were housed separately in plastic cages under controlled condition of temperature and light. The animals were divided into 3 groups Control, Sham and Experimental. The sham group was given the normal food in low quantity whereas control was given normal food in sufficient amount. The experimental mice were given food pellets (Hindustan Lever Pvt. Ltd.) were exposed to microwave radiation in microwave oven at 320° C for 10 minutes. The experimental group was administered with fixed amount of microwave cooked mice pellets daily for 2 weeks (Experiment 1), 3 weeks (Experiment 2), 4 weeks (Experiment 3). The recovery group (Experiment 4) was given the microwave pellets for 4 weeks and after that they were given normal mice fed for 4 weeks. After the termination of each of experimentation group, the treated and control males were sacrificed by cervical dislocation and the testis, seminal vesicle and prostate were collected in 0.1% NaCl. The body weight and organ weight (testis) was recorded at each intervals.

Sperm Count: A small aliquot of sperm suspension is diluted with sperm diluting fluid and counted on a hemocytometer. This count is used to calculate the total number of sperm per epididymis.

Sperm Morphology: Sperm abnormality was tested according to the method of Wyrobek *et al.* [7]. The epididymes were excised and minced with fine scissors in physiological saline in a petri dish. Smears were made on clean, greasefree slides with a mixture of normal saline (9:1) for 45 min. The slides were air-dried and stained with Papanicolaou stain for subsequent examination under microscope and different abnormalities were recorded. Cytological evaluation for sperm abnormalities was carried out using a binocular microscope. The following categories of defects were scored: Head shape/size defects or double heads, or any combination of these. Neck and midpiece defects, including absent tail, non inserted or bent tail, abnormally thin midpiece or any combination of these. Tail defects, including short, multiple, broken, irregular width, coiled tails, tails with terminal droplets, or any combination of these and cytoplasmic droplets greater than one-third of the area of a normal sperm head.

't' test was used to determine the significance value of the experimental data.

RESULT

Gonadotrophic ratio is expressed as ratio of organ weight to body weight. Significant decreases in gonadotrophic ratio of testis was observed (Table 1). After feeding of microwave exposed food for 2 weeks and 4 weeks, there is a highly significant decline in the level of organ to body weight ratio in experimental group and sham group as compared to control ($p < 0.01$) The significant recovery ($p < 0.01$) was shown by the recovery group

There is significant decline by $p < 0.01$ in the sperm count of animals, fed on microwave exposed food, for 2 weeks and 3 weeks and 4 weeks and sham group as compared to control. In Experiment group 4 the value of sperm count increased and become comparable to the control.

The level of deformities continuously increased with increasing exposure duration. (Table 2) The various abnormalities that were observed were coiled tails, oval head, cytoplasmic droplet, bifurcated tail (Figure 1-6). The number of tail deformities particularly cytoplasmic droplet was higher than rest of abnormalities.

The changes in the value of control within experimental group are attributed to increasing age of the animal.

Table 1 : Effect on Gonadotrophic ratio and Sperm Count of Swiss Albino Male Mice fed on Food Exposed to Microwave Radiations.

Parameters	2 weeks			3 weeks			4 weeks			4 weeks recovery		
	C	S	E	C	S	E	C	S	E	C	S	E
Gonadotrophic Ratio	0.0061±0.0056	0.0027 ± 0.0001***	0.0024±0.0002**	0.0074±0.0006	0.004±0.0003***	0.0038±0.001**	0.0071±0.0003	.0058±0.001**	0.004±0.003**	0.0013±0.0009	0.00292± 0.0009***	0.00396±0.0002**
Sperm count in 10 ⁶ /ml per caudaepididymis	.36 ±.10	.18±0.014**	.17 ±0.03**	.63 ±0.017	.44 ±0.048**	.49 ±0.01**	0.64 ±0.028	.45 ±.049**	.473 ±0.01**	.617 ±0.013	.452 ±0.04**	0.492 ±0.009**

C (Control); S(Sham); E(Experimental)
Significance in relation to control*p<0.05, **p<0.01

Table 2: Effect on Sperm Morphology of Swiss Albino Male Mice fed on Food Exposed to Microwave Radiations.

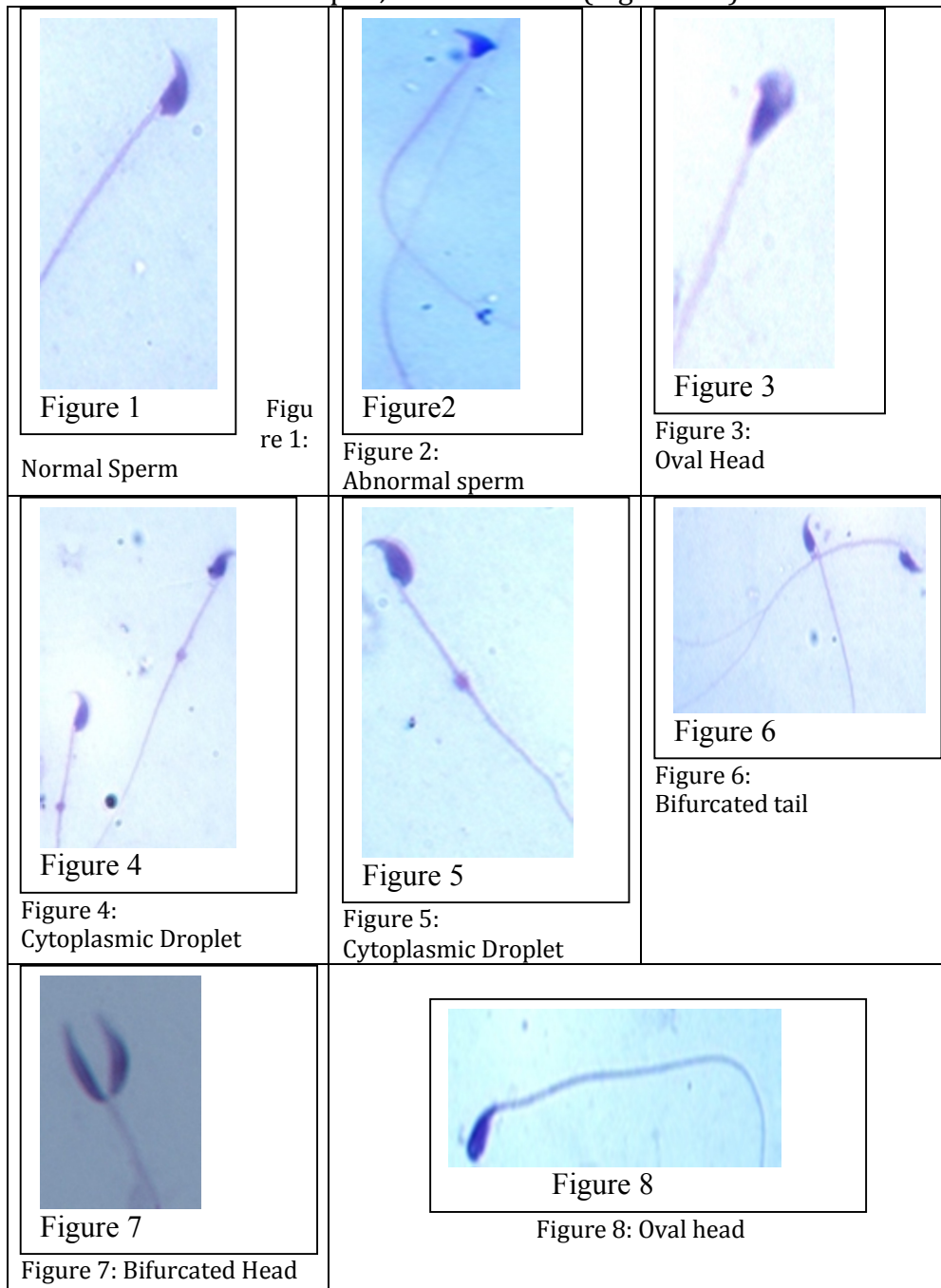
Autopsy Interval	Groups	Normal	HEAD DEFORMITY (%)	NECK DEFORMITY (%)	TAIL DEFORMITY (%)
2 week	Control	77.6	4.8	7.4	10.2
	Sham	63.1	7.3	11.3	18.3
	Experimental	45.8	11.4	20.4	22.4
3 week	Control	74.2	7.3	8.0	10.5
	Sham	48.5	9.3	20.1	22.1
	Experimental	36.7	14.2	22.6	26.5
4 week	Control	70.1	10.0	7.1	12.8
	Sham	37.9	21.3	20.4	20.4
	Experimental	36.0	18.3	20.05	25.65
4 week recovery	Control	65.2	12	10.3	12.5
	Sham	35	23.5	21.5	20.0
	Experimental	42.5	19.3	18.1	20.1

DISCUSSIONS & CONCLUSION

In the present study a general decline in the gonadotrophic ratio of testis as well as suppression of spermatogenesis was noticed in the animals fed on microwave exposed food for 4 weeks; however, complete cessation of spermatogenesis did not occur as evidenced by the presence of sperm population. The drastic decrease in the gonadotrophic ratio of testis may be due to absence of spermatids and spermatozoa from the testes and accessory organs as demonstrated by Paul *et al.* (8). The change in testicular weight has also been found to correspond to the presence or absence of sperm in testis. The weight of testis is known to be a good index of FSH secretion. The reduction causes a significant decrease in the weight of testis and accessory organs in the male. It was clearly observable that there is a decline in sperm count with increasing duration of feeding of microwave exposed food. Reduced sperm number reflects the antiandrogenic nature of the microwave exposed food. Since the analysis of sperm parameter pertained to the cauda epididymal spermatozoa changes indicate the effect of microwave exposed food at the level of sperm

maturation. A decrease in sperm count may be due to death and removal of the sperm during cauda epididymal stage.

The various abnormalities that were observed were coiled tails, oval head, cytoplasmic droplet, bifurcated tail (Figure 1-6).



Decrease sperm count, increase incident of sperm abnormalities strongly point to a spermatotoxic effect of microwave cooked food, particularly coiled tail nature of sperm suggest some biochemical changes in sperm surface [9]. The cytoplasmic droplet (CD) is a smear of cytoplasm initially remains attached to the neck region and gradually shift its position to the end of the mid piece during epididymal transit of the sperm. The droplet is shed when the sperm leaves the caput epididymis and when sperm arrives at the cauda they are devoid of droplet. The sperms which retain extra cytoplasm are inhibited in motility [10-11]. The increase retention of cytoplasmic droplet by cauda epididymal sperm with the increased duration of food administration would be speculated as

microwave food impairing the process of shedding of cytoplasmic droplet. The impact on sperm morphology indicates the impairment of sperm processing in the epididymis.

In the male reproductive system, reduced sperm count and increased sperm abnormalities are among standard criteria used to characterize toxic agents that may cause fertility problems in treated subject. [12].

In conclusion, this study shows changes in sperm count, sperm morphology, of young-adult animals fed with microwave exposed food for different duration. Overall, our results confirm that different duration of feeding of microwave exposed food play a role in alteration of sperm parameters, which are recoverable by the substitution of microwave food by normal food.

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