

## Effect of N-Methyl N-Nitrosourea Induced Cancer on the Fertility of Female Albino Wistar Rat

Adedokun Adewale Anthony<sup>1</sup> Adetokun Isreal Olufemi<sup>2</sup> Rajideen Saheed<sup>3</sup>

<sup>1,2,3</sup>federal Polytechnic, Kaura Namoda

\*Corresponding author, Email Address: [awaledokun@gmail.com](mailto:awaledokun@gmail.com)

**Abstract:** The present study was designed to understand the effect of multiple dose of N-methyl-N- nitrosourea (MNU), an end product of methylguanidine, in albino rat cancer induction. This is borne out of the fact MNU which is reportedly found in many processed stored food has been traced to the incidence of cancer in humans recently. In this research, a total of five experimental groups of animals (albino Wistar rats) were taken, considering group-I as vehicle control and group-II-V as induced groups (where group-II-V were induced with single to quadruple doses of 50 mg/kg of MNU respectively in a three weeks interval). After the accomplishment of MNU injection, each rat was mated with a fully grown healthy adult male rat to check the fertility efficiency of each group in terms of pregnancy rate. The results of the study indicated that the rat group induced with the highest dosage of MNU was 33.3% to 57.2% less efficient in being impregnated than the rats in the control group. There were observed changes in body weight, and vital female organs like the uterus and ovary upon treatment with MNU compared to the control group with the trend of increase proportional to the increasing dosage of MNU compared to rats in the control group. These observed changes in the MNU-induced groups may be attributed to the resultant effect of the chemical which causes alkylation of DNA components resulting in hormonal disturbances in the body of rats.

### 1. Introduction

In recent years various reports have been published on the role of food carcinogens in increased cancer incidence and infertility in the human population (Clark *et al.*, 2019). Methylguanidine, an originator of MNU which is highly carcinogenic has been detected in many animal meats and processed stored food often in high concentration (Fujinaka *et al.*, 1976). The carcinogens might not be directly present in raw food materials but the presence of some originators like methyl guanidine promotes the production of MNU like carcinogens during processing or storage (Fujinaka *et al.*, 1976). N-nitroso compounds are known food carcinogens and are easily produced by reaction between the oxide of nitrogen and secondary or tertiary amine (Janice *et al.*, 2009). On the other hand exposure to these carcinogens causes hormonal disturbances in the body along with severe reproductive problems and other health disorders. Human population is generally exposed to these compounds through diets like preserved meats, smoked fish, sausages, dried milk, pickles, etc. (Liu *et al.*, 2009). People working, as hairdressers, in rubber industries, with metal, leather, and fertilizer are also prone to get contaminated with these carcinogens (Lewis *et al.*, 2013).

There are also chances of getting exposed through cosmetics and with few pharmaceutical products as well. (Carpenter and Bushkin-Bedient, 2013). N-methyl-N-nitrosourea (MNU) is one such carcinogen found in various processed food materials in significant amounts (Donnelly *et al.*, 2004). MNU serves as an alkylating agent that damages DNA by binding to it which causes genetic mutation and ultimately increases the risk of cancer incidence and progression (Gorbachev *et al.*, 1988). MNU-induced retinal degeneration is popular in the study of the therapeutic trials (Tsubura *et al.*, 2011) A single dose intraperitoneal injection (50 mg/kg body weight) of MNU in Sprague Dawley (SD) rats was found to induce (ER+) dependent breast tumor (Rivera *et al.*, 1994). Also, MNU-treated retinal degeneration and/or degenerative disease models are very popular to study therapeutic trials (Chen *et al.*, 2014). The altered organotropic effect of MNU in different animal models depends not only on dose but also on route, age, and sex (Chang *et al.*, 2012). The hormonal imbalance treated by MNU might affect fertility by damaging the reproductive organs.

Considering the above factors, this research is aimed at studying the multiple effects of MNU-induced on the reproductive system using female Albino Wistar Rats as a case study and thereafter the effect of MNU on developing cancer incidence in female vital organs.

## **2. Materials and Methods**

Female Albino Wistar rats used were approximately 9 weeks old and weighed approximately 20 g. The rats were obtained from the National Veterinary Research Institute (Vom near Jos in Plateau State of Nigeria). Rats were housed in suspended, stainless-steel, wire-bottom cages. Environmental controls for the animal rooms were set to maintain a temperature of 64°C to 79°C. The N-methyl N-nitrosourea (MNU) used was obtained from Sigma Chemical Co. Ltd. Kano.

### **Induced MNU Treatment of the Female Albino Wistar Rats**

The animals were randomly divided into 5 groups of 6 in each group. Group I served as the control group while groups II, III, IV, and V were induced with single to quadruple doses of MNU. The MNU to be induced containing 50mg/Kg body weight of the rat was dissolved in 0.9% normal saline (Raghuram *et al.*, 2021). This was administered intraperitoneally (IP) for better absorption into the animal's body system at fixed time intervals (Das *et al.*, 2019 Samanta *et al.*, 2016)

- To determine the effect of MNU at different exposures in Albino Wistar Rats, the change in body weight was recorded weekly.

The mating procedure of Male rats with Albino Wistar rats

After 8 weeks of observation of the female Albino Wistar rats, a matured male rat was introduced in each subdivided group for a chance of pregnancy and breeding process (Kandimalla *et al.*, 2021). The male rats were randomly shifted within the sub-divided groups

on alternative days to increase the chance of pregnancy. After 4 weeks of observations, the percentages of pregnant rats per group were recorded.

### Dissection and isolation of the reproductive system of the Rats

Two weeks after giving birth, the pulp and mother were adequately taken care of and 3 mother rats were randomly separated from each group, dissected and the reproductive system isolated for observation (Kandimalla *et al.*, 2021). This is to see the effect of MNU on the reproductive system.

## 3. Results and Discussions

### Effect of MNU on physiological changes

Fig 1 shows body weight change in the Albino Wistar rats treated with single to multiple doses of MNU over a period of 12 weeks. Observation showed that all groups gained weight slightly in the first 3 weeks while animals of the control group (Group-I) gained weight concurrently to more than that of the MNU-treated rats of Group-II to Group V after week 3. The relative weight gain reduces until the final week in all MNU-induced groups. This establishes that MNU has no consistent effect on the body weight but weight increase in MNU-induced rats is relatively smaller compared to weight increase in the control group over the period of study.

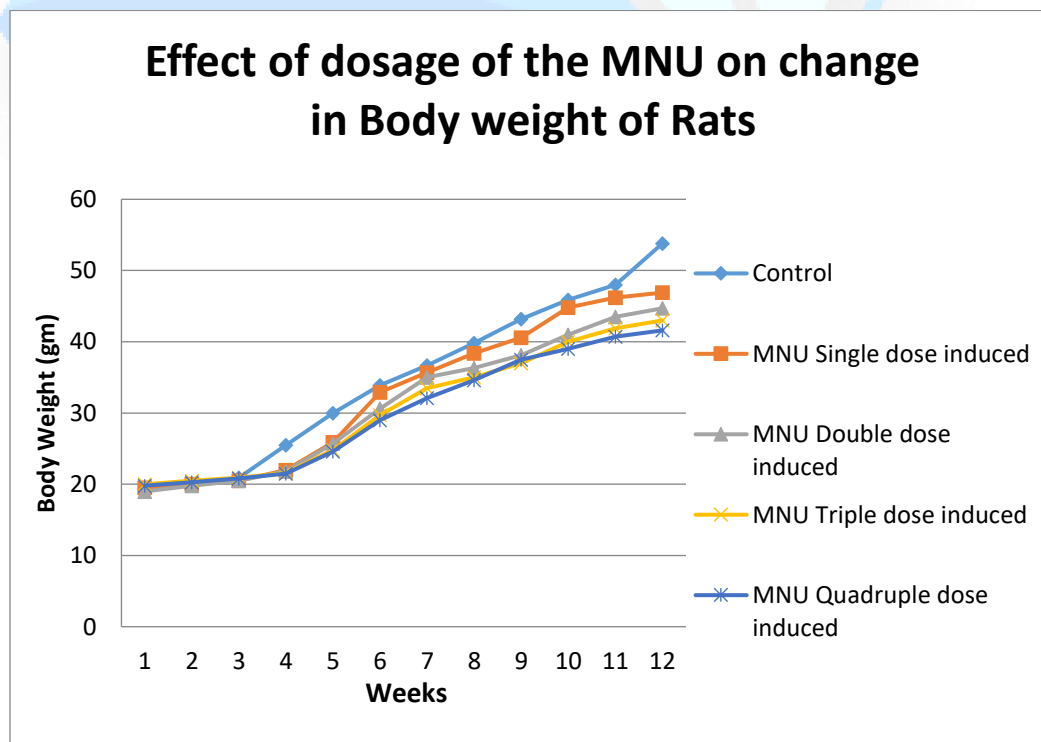


Fig 1: Effect of N-methyl N-nitrosourea Dose on Body Weight of Rats

Food Intake

The average food intake by rats per group induced with various doses of MNU over the period of study was shown in Fig 2. It was observed that the food intake of each MNU-induced group II to V were relatively lower than that of the control group I. whereas among the different MNU-induced groups, overall food intake record become lower as the MNU dose is increase. The observed reduction in the food intake might as well be responsible in reduced weight gain noticed MNU-induced compared to the control group as shown in Fig 1.

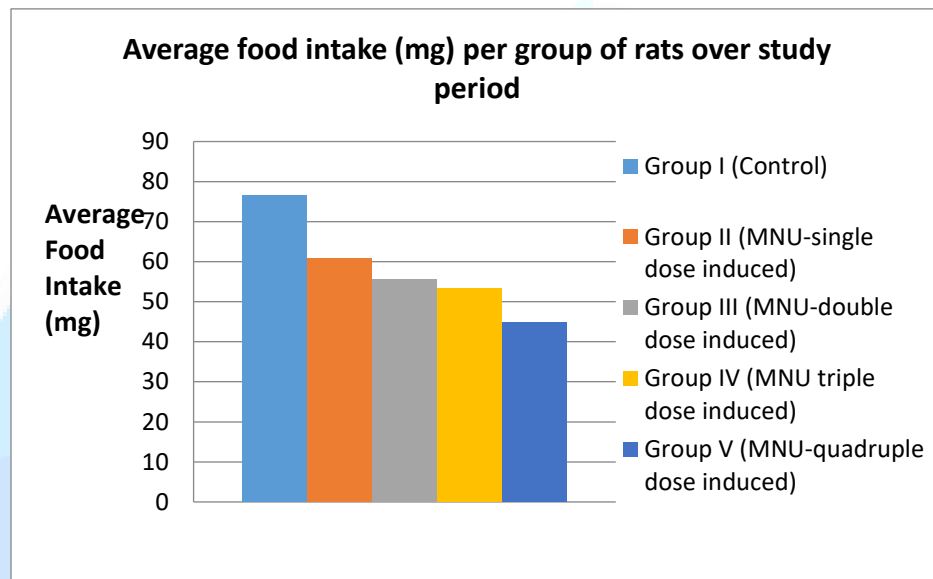


Fig 2: Effect of MNU dose on food intake per group of Rats

### Effect MNU on Chance of pregnancy of the Albino wistar Rats

As revealed in Table 1, the results showed that the percentage pregnant of female Albino wistar rats in group I was significantly higher compared to that of MNU- induced groups. The results show a significant effect of MNU dosage on chance pregnancy of the induced rats. The pregnancy rate is reduced as the induce dose of MNU is increased from group II with single dose of 50mg/Kg body weight of rat to quadruple dose in group V. This suggested that 8 weeks of exposure to MNU before breeding process commenced and after weeks may have affected the reproductive system of the rats thus leading to reduced chance of pregnancy.

Groups	MNU dose (50mg/Kg)	Pregnancy rate (%)
I	Control	100
II	Single	66.7
III	Double	50.0
IV	Triple	45.5
V	Quadruple	42.8

### Effect of MNU on the reproductive organ of the Rats

The observed weight of the ovary and uterus combine increases as number of MNU-induced dose increase Fig 3. While the mean weight of the combine ovary and uterus of the control group stands at 0.26 g/rat, that of the MNU-induced rat increases between 0.38 g to 0.45 g/rat.

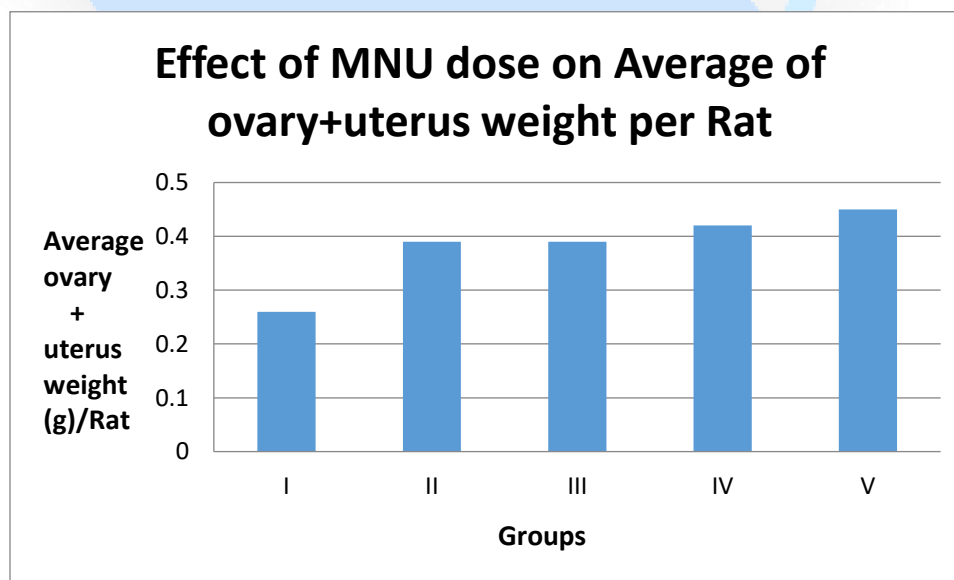


Fig 3: Effect MNU on the Reproductive system of the Rats

#### 4. Conclusion

The MNU exposure rats losses their efficacy to get pregnant as much about 57% lower compared to untreated MNU group. Although the data was not statistically significant because of either lower sample size or lesser experiment time, but the clear trend is there in lowering the efficacy of getting pregnant. The present study, have evaluated the effect of multiple exposure of lower dose (50 mg/kg) of MNU in Albino Wistar Rats for fertility or cancer induction in female vital organ and to understand the long term and/or effect of multiple exposure of MNU to cause serious health issues like, infertility, cancer induction, etc. in Albino Wistar Rats. MNU is noted here to causes DNA disorder through its alkylating ability (Kandimalla *et al.*,2021)

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