

Effects of injected N-Methyl-N-Nitrosourea (MNU) in albino mice on the histology and haematology of selected organs of the circulatory, lymphoid and digestive systems

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ABSTRACT

Aims: This study was performed to assess the effects of N-methyl-N-nitrosourea (MNU) on the hematology and histology of selected organs in albino mice.

Study design: Eighty male and female mice, eight-week old, were subdivided into four groups of twenty each.

Place and Duration of Study: Department of Zoology, University of Ibadan between January and July 2011.

Methodology: Forty male and female mice received a single dose of 50mg/kg MNU in 1mL of 0.9% NaCl via intra-peritoneal injection. One mouse from each group was sacrificed each month and processed using standard histological techniques. At the end of the study, blood samples collected from two mice per group were processed using standard hematological procedures.

Results: MNU induction impacted different morphological abnormalities in induced mice: vacuolization (small intestine), multi-nucleation (spleen, liver), cytoplasmic pallor (heart, liver), increased lymphocytic activity (spleen, thymus), necrosis (heart, liver), and destruction of intestinal wall (small intestine). Increased nuclear-cytoplasmic ratio, nuclear pleomorphism, enlarged nuclei sizes and cytoplasmic degeneration were also common features observed. One of the induced mice developed a neck tumor eight weeks after induction. The histological section of the tumor showed complete necrosis. Except for increased nuclei sizes in the liver, these abnormalities were not observed in control mice.

Hematological analysis did not show any significant differences ($p=0.05$) in packed cell volume, hemoglobin, red blood cell count and monocytes between the induced and control mice.

Conclusion: Exposure to MNU over a 28-week period caused varying degrees of

abnormalities in induced mice. The study duration however appeared insufficient for the development of any pronounced hematological effects in induced mice.

Keywords: [Carcinogenesis, Histopathology, Hematology, N-methyl-N-nitrosourea, Swiss Albino mice, Tumors].

1. INTRODUCTION

Chemically induced carcinogenesis models in animals especially rats and mice are widely used for studying the biology of cancer and for developing and evaluating cancer treatment strategies. N-methyl-N-nitrosourea (MNU) is one of the widely used chemicals in several studies of chemical carcinogen models. It has been predominately used in tumor induction and in the investigation of a variety of novel chemo-preventive and treatment agents (Macejova and Brtko, 2001). The works of several authors have established MNU as a potent carcinogen in the induction of diverse tumors on varying strains of rats and mice. Grubbs et al., (1981) examined its effects on hamsters. The induction of mammary tumours in Sprague-Dawley rats has also been well reported (Moon et al., 1992; Rivera et al., 1994; Takahashi et al., 1995 and Thompson et al., 1995). Franchi et al., (2003) examined its effects on Wistar rats, while its effects on the hematology of Wistar rats were reported by Akanni et al., (2010).

However, there is paucity of information as regards its effects in the Swiss albino mice strain, and thus its susceptibility and suitability in carcinogenesis models involving MNU. Differences in susceptibility may occur across different strains, which is indicative of genetic variation in response (Satoru et al, 1985; Festing, 2010). In particular, differences may occur across strains in the target organs that may be affected by MNU. For instance, while MNU induced mammary gland carcinomas in Sprague Dawley rats, (Moon et al, 1992; Rivera et al, 1994; Thompson et al, 1995); thymic lymphomas were observed in Wistar rats and P53+/- mice. Splenic hemangiomas and small intestine adenomas were also reported respectively (Franchi et al, 2003; Morton et al, 2008). MNU was also found to induce cataracts in 0-20 day old Sprague Dawley rats (Yoshizawa et al, 2000). The time required for the effects to be observed is another important factor. The objective of this two part study (the first of which is reported here), is therefore to provide information on the effects of MNU induction in the Swiss albino mice strain. Furthermore, reports on the hematology of tumor bearing rats particularly in Swiss albino mice appear to be scarce. Hence this study also seeks to provide information in this regard.

Although there have been variations in the dosage of MNU administered, the use of a single dose of 50mg/kg has been used to induce carcinomas by most authors (Rivera et al, 1994; Thompson et al, 1995; Pazos et al, 1998). This dosage is ideal for applications in which rapid tumor induction with high yield is desired (Thompson and Adlakha, 1991). This thus informed the use of this dosage in our study. To achieve the objective stated above, a histological evaluation of selected organs of the circulatory (heart), lymphoid (thymus, spleen) and the digestive systems (liver, and small intestine) in mice given a single intra-peritoneal dose of MNU were carried out. Additional information on the effects of the MNU on some haematological parameters [Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC), White Blood Cell (WBC) and its differentials (Lymphocytes, Neutrophils, Monocytes and Eosins), Platelets and Plasma] in the mice was also evaluated.

66 2. MATERIAL AND METHODS

67

68 2.1 Experimental animals.

69 Eighty (80) eight week old albino mice made up of forty males and forty females were
70 used for this study. The mice were obtained from the Department of Anatomy, College of
71 Medicine, University of Ibadan, Ibadan, Nigeria. They were later housed ten each in iron
72 cages with wooden chips for bedding in a well-ventilated room and maintained on 12 h light /
73 12 h dark cycle in the animal house of the Department of Zoology. These mice were fed with
74 standard laboratory diet and had access to water *ad libitum*. They were acclimatized for a
75 period of two weeks prior to the commencement of the experiment.

76

77 2.2 Chemical carcinogen.

78 N-methyl-N-Nitrosourea (MNU Sigma Chemical Co., St. Louise Mo, Catalogue No. 684-
79 93-5) was purchased from Sigma Aldrich and was used to initiate the carcinogenesis
80 process.

81

82 2.2.1 Preparation mode for the carcinogen

83 A single dose of 50mg/kg of MNU was administered to the mice via intra-peritoneal
84 injection. The carcinogen (10mg) was dissolved in 1ml of 0.9 % sodium chloride. Based on
85 the average weight of 24.47g and 20.14g for males and females respectively, the mice were
86 thus given a calculated dosage of approximately 0.1ml MNU for both the males and females.

87

88 2.3 Experimental Design

89

90 2.3.1 Induction with the carcinogen MNU

91 Mice of both sexes were randomly divided into four groups of twenty each (MI, FI,
92 MC and FC). Groups MC (male control) and FC (female control) served as the control
93 (members of this group were not induced nor given placebo) while groups MI, (male
94 induced) and FI (female induced) received 0.1ml of MNU via intra-peritoneal injection.
95 After the carcinogen induction, the mice were kept on basal diet and water *ad libitum* and
96 were subsequently weighed weekly to monitor changes in their weight. From the second
97 week after induction, animals were palpated twice weekly to monitor possible tumor
98 appearance. Complete gross examination was carried out for detection of tumor. The
99 animals were monitored for 28 weeks. One mouse from each of the four groups was
100 sacrificed for histological examination at the end of every four weeks. In all, a total of
101 twenty eight mice comprising seven mice from each of the four groups were
102 sacrificed over the 28-week study period and processed for histological examination.
103 In addition, a tumor bearing mouse which died before the end of the study period was
104 also processed for histological examination, bringing the total number of mice
105 examined to twenty nine. The mice were anaesthetized with chloroform (except for the

dead mouse) and subsequently dissected to obtain the selected organs: liver, spleen, thymus, small intestine and heart, which were then stored in 40% formal saline prior to histological analysis. Histological analysis was carried out using the following equipment: a Leica rotary microtome model 2125 RT, a Leica tissue embedding machine model eg 1160, a Raymond Lamb tissue floatation bath model E652 and hot plate model E18, as well as a Tissue-Tek II tissue processor model 4634. The prepared slides were viewed using an Olympus CX31 microscope with computer attachment. **Results of representative slides are however presented in this paper. Except for the sacrificed animals, all other mice were monitored weekly for changes in their body weight, hence total number of mice in each of the four groups (n) varied from 20 at the beginning of the study period, to 13 mice at week 28 respectively. The male induced group (MI) however contained 12 mice instead of 13, due to mortality in the tumor bearing mouse. The remaining mice were used for the second part of this study (not reported here).**

2.3.2 Collection of blood samples and measurement of hematological parameters

At the end of the 28-week study period, two mice from each of the four groups (eight in all) were sacrificed out of the fifty one mice remaining. This was to provide some preliminary information on the probable effects of the carcinogen on hematological parameters. **However, only two mice per group were sacrificed for this purpose. This was to allow for an adequate sample size for the second stage of this study which is currently in progress.** Blood samples were collected from the retro-orbital sinus of the mice with heparinised 70ml micro-haematocrit capillary tubes into a vial containing 0.5ml Ethylene Diamine Tetra Acid (EDTA). White blood cell (WBC) counts, RBC counts, PCV, platelets and hemoglobin (Hb) content of the blood obtained from the mice were analyzed at the hematology unit of the Department Of Veterinary Medicine, University Of Ibadan, Nigeria using the method of Moore, (1958) and Stevens, (1997).

2.3.3 Statistical Analysis.

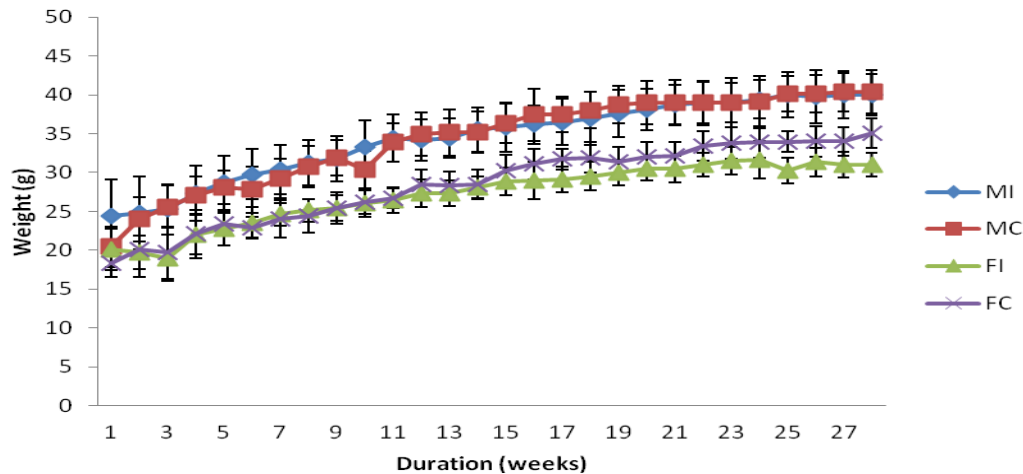
T-Test was used to determine if there were any statistically significant differences (at $p = 0.05$) between the means of the control and test group for each of the hematological indices determined, as well as for the body weight. Sexes were analysed separately.

3. RESULTS

3.1 Body Weights and Clinical Signs

Members of both groups gained weight progressively during the study period (Figure 1), with the induced and control males reaching a maximum weight of 40.03g and 40.37g respectively, at the end of the study period. The female control group presented a mean body weight ($\pm$ SD) of $28.48g \pm 0.95$, which was higher than the mean body weight of the female induced group ($27.24g \pm 0.73$). However this difference was not significant at $p = 0.05$. **Furthermore, the average weight of the induced male mice ($34.38g \pm 0.94$) was higher than the male control mice ($34.23g \pm 1.08$) by a difference of 0.15g, which was**

also not statistically significant at $p = 0.05$. The induction with MNU appears not to have caused any changes in the behavior of both the control and the induced mice. Majority of the induced male and female mice appeared to be healthy and showed no observable behavioral differences from their respective control groups. The induced mice also did not show any form of labored or rapid breathing patterns. One of the male induced mice was suspected to have developed a tumor in the cervical region barely eight weeks after the carcinogen induction. Except for the mouse with tumor, no mortality was observed in the induced or control mice.



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160

161 Fig. 1. The average weight of both the induced and control albino mice over a 28-week study
162 period.

163 *Mean $\pm$ S.D = Mean values $\pm$ Standard deviation of twenty mice per group.*

164 *However there was variation in N over the study period due to the exclusion of animals sacrificed for histology at the*
165 *end of every four weeks (seven in each group over the study period). One mouse also died in the group MI before*
166 *the end of the study period.*

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168 3.2 Histopathology.

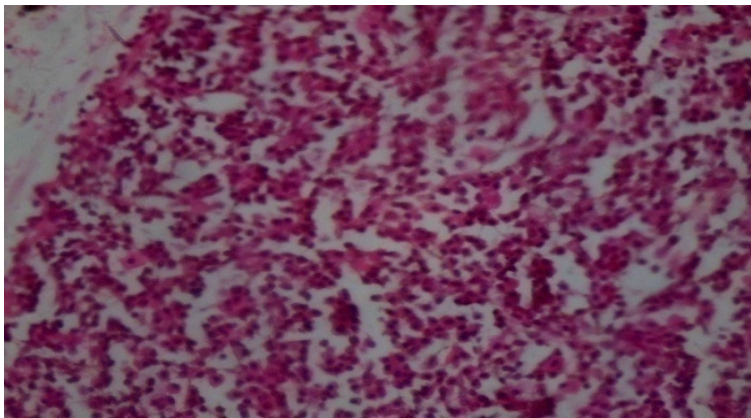
169 There were varying degrees of lesions and abnormalities in the induced animals when
170 compared to the control. Abnormalities were observed in all the seven induced male
171 and female mice respectively, which were sacrificed over the study period. Except for
172 increased nuclei sizes in the liver, all the control mice sacrificed (seven in each
173 group) did not record any abnormalities or lesions. Representative figures are
174 however used here for the comparison. The figures presented represent some of the
175 abnormalities observed in the organs of different mice examined during the study
176 period. Figure 2 shows the histological section of the thymus of a female control mouse
177 with normal architecture and well delineated cells. Vesicular nuclei, nuclear pleomorphism,
178 increased nucleo-cytoplasmic ratio and increased lymphocytic activity (Figure 3) were some
179 abnormalities observed in the thymus of the induced mice. While normal architecture with
180 well delineated striated muscles was observed in the heart of the control mice (Figure 4), the

181 morphological changes observed in the heart of the induced mice included nuclear
182 pleomorphism with prominent nucleoli and necrosis (Figures 5 and 6).

183 The spleen of the control showed normal architecture (Figure 7), while some of the
184 abnormalities observed in the induced mice were: multinucleation (Figure 8), cytoplasmic
185 degeneration, increased lymphocytic activity and nuclear pleomorphism (Figure 9). The liver
186 of the MNU treated mice (Figures 11-12) also showed some of the above mentioned
187 abnormalities. These included necrosis and cytoplasmic degeneration associated with
188 cytoplasmic pallor (Figure 11), multinucleation, increased nucleo-cytoplasmic ratio and
189 pleomorphic nucleus infiltrating the stroma (Figure 12). There were also dilation of the
190 sinusoids, and disintegration of hepatic cells including necrosis of hepatocytes, and enlarged
191 nuclei in the liver of the treated mice.

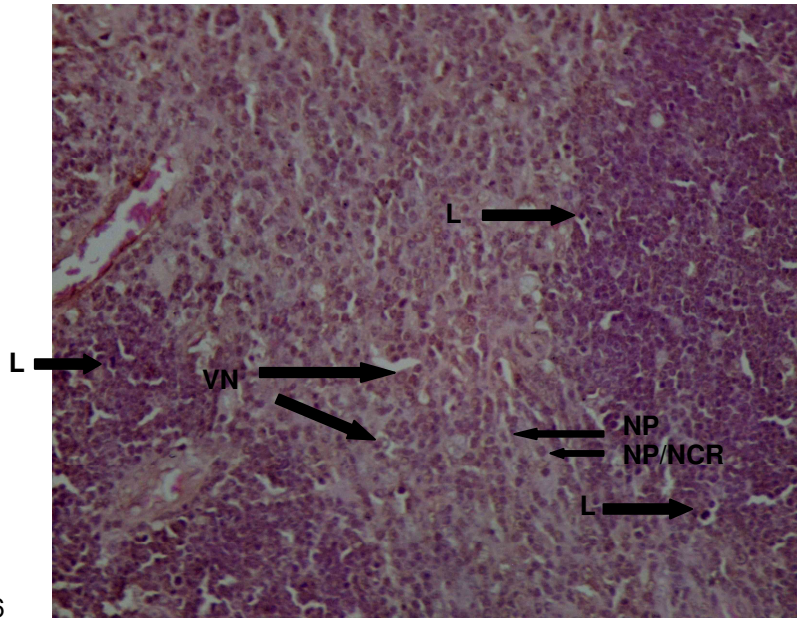
192 Other observable features were; serious disarrangement and degeneration / distortion of
193 the regular hexagonal shape of the hepatocytes. Furthermore, most of the cytoplasm have
194 lost their eosinophilic colours, the radial architecture of the cells has also been altered and
195 the cells thus lack distinct outline and appear reduced in size indicating distortion of the
196 hepatic architecture. Some of these features were however, observed in the histological
197 sections of the control mice (Figure 10). The histological sections of the small intestine of the
198 control mice showed well defined architecture with unaltered intestinal wall (Figure 13).
199 However, degeneration of the intestinal wall of the small intestine and vacuolation were
200 noticed as illustrated in Figures 14 and 15. The histological section of the cervical tumor
201 observed in one of the exposed mice barely eight weeks after exposure showed complete
202 necrosis (Figure 17).

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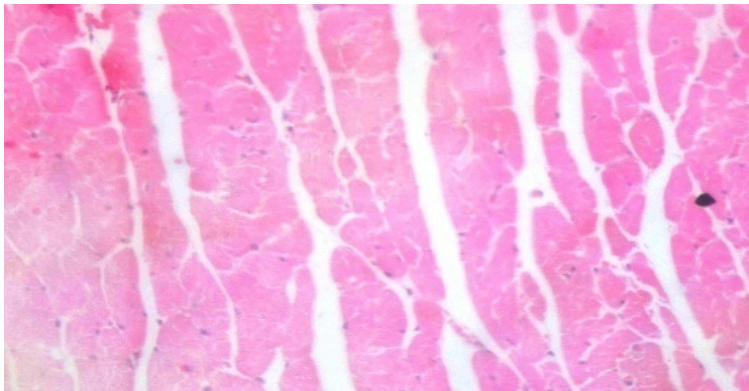
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205 Fig.2. Control Thymus Female showing normal architecture. (H & E X 40).



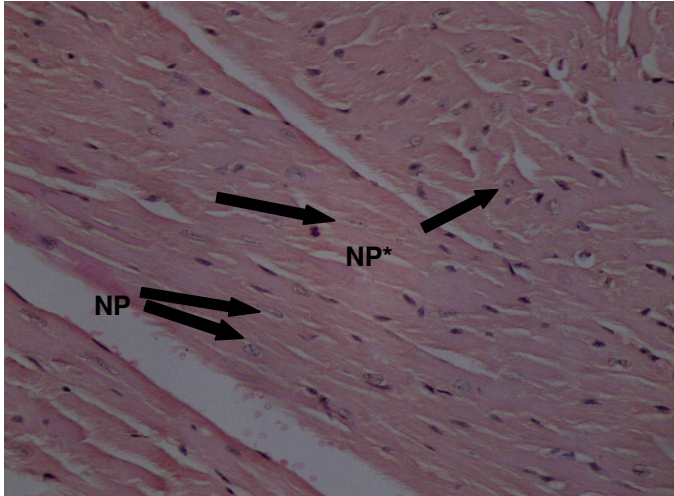
206

207 Fig.3. Induced Thymus Female showing Vesicular Nuclei (VN), Nuclear Pleomorphism (NP),
 208 increased Nucleo-Cytoplasmic Ratio (NCR) and increased lymphocytic activity (L) (H&E X
 209 40 obj.)



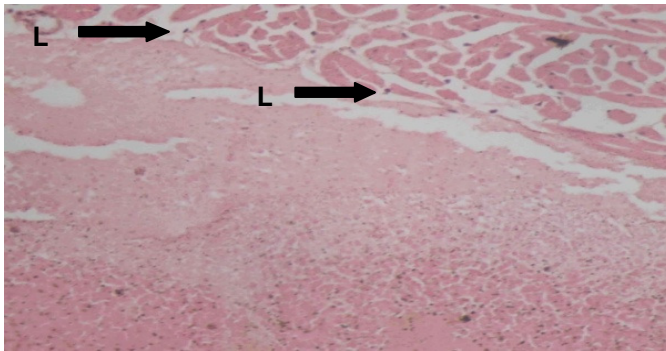
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211 Fig.4. Heart Control Male showing normal architecture with well delineated striated muscle
 212 and evenly distributed cardiomyocytes (H&E X40 obj.)



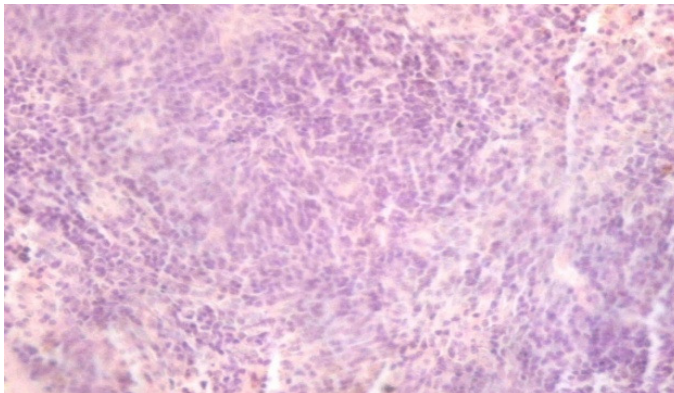
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214 Fig.5. Heart Induced Male Showing Nuclear Pleomorphism (NP) and pleomorphic nuclei with
 215 prominent multiple nucleoli (NP*) (H&E X40 obj.)



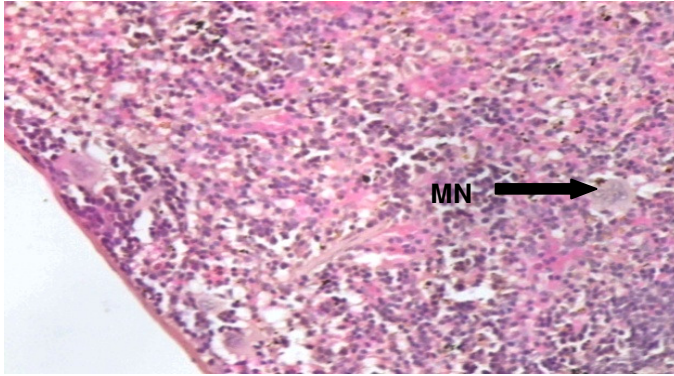
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217 Fig.6. Heart Induced Male showing Necrosis and scanty lymphocytes (L) (H&E X40 obj.)



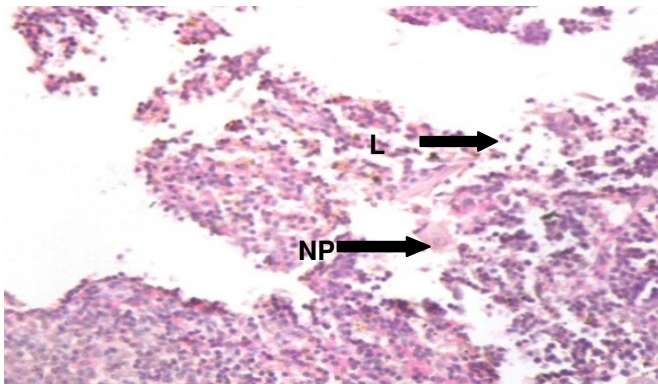
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219 Fig.7. Spleen Control Male showing normal architecture (H&E X 40 obj.)



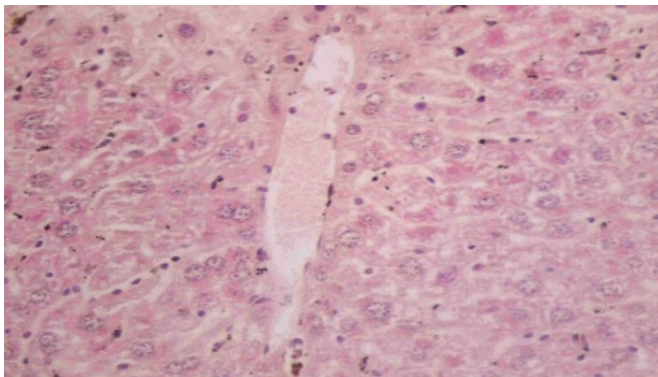
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221 Fig.8. Spleen Induced Male showing Multinucleation (MN) of the cell (H&E X40 obj.).



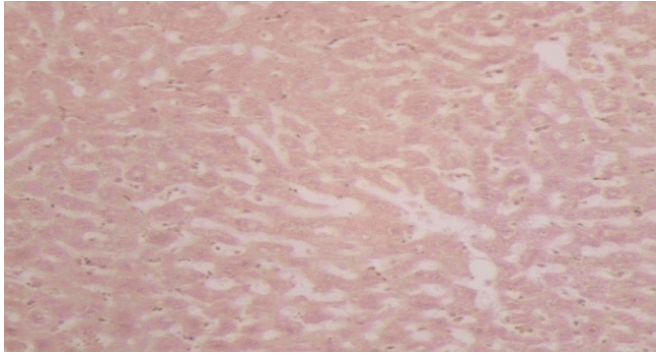
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223 Fig.9. Induced Spleen Male showing Cytoplasmic Degeneration(CD), increased Lymphocytic
224 activity (L) and Nuclear-Pleomorphism (NP). H&E (X40 obj.)



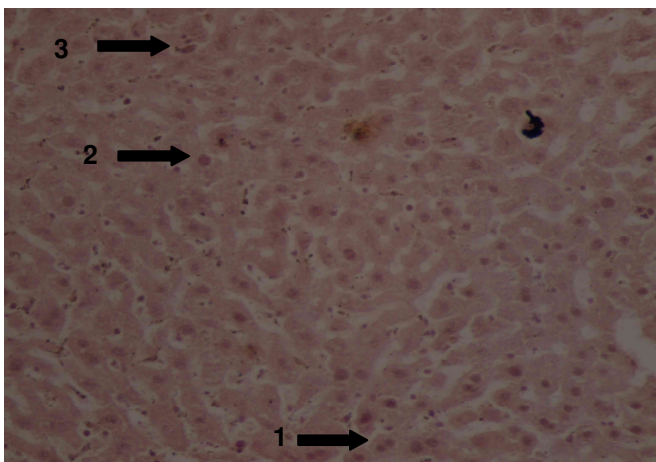
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226 Fig.10. Histological section showing normal appearance of female liver (H&E X 40 obj.)



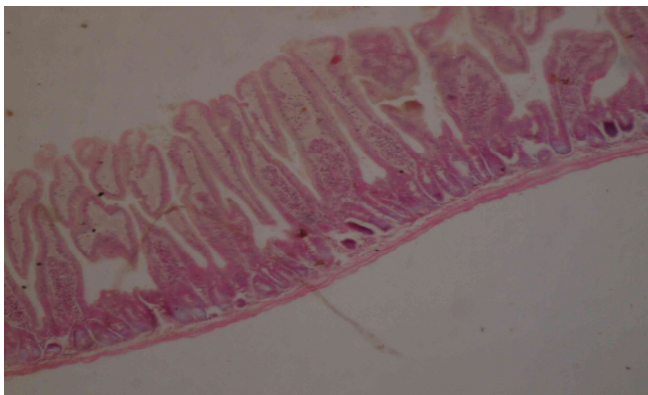
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228 Fig.11. Histological section of the liver of an induced female showing necrosis and
229 cytoplasmic degeneration associated with cytoplasmic pallor (H&E X 40 obj.)



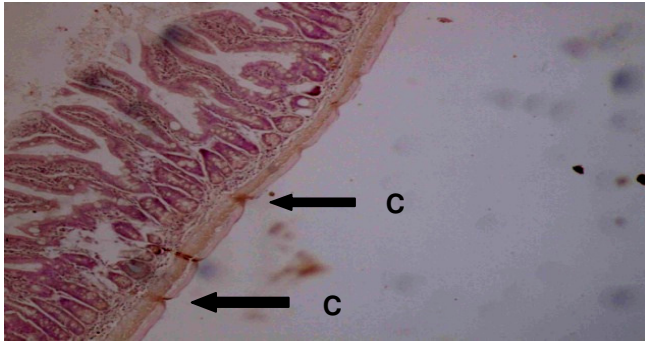
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231 Fig.12. Histological section of the liver of an induced female showing (1) multinucleation, (2)
232 increased nucleo-cytoplasmic ratio and (3) pleomorphic nucleus infiltrating the stroma (X 40
233 obj.)



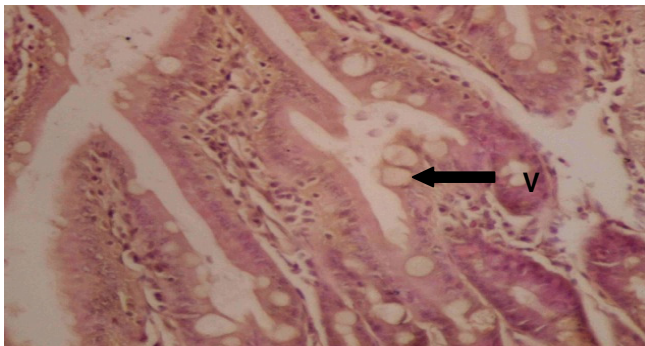
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235 Fig.13. Histological section of the small intestine of **male** control mice showing normal
236 architecture and unaltered intestinal wall (**H&E X 10 obj.**)



237

238 Fig.14. Histological section of the small intestine of an induced **male** showing corrosion of
 239 the intestinal wall (C) **(H&E X 10 obj.)**



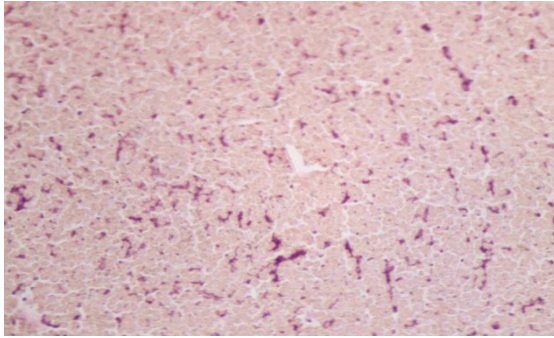
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241 Fig.15. Section of the small intestine of an induced **male** showing presence of numerous
 242 vacuoles **(H&E X 40 obj.)**



243

244 **Fig.16. Suspected tumor in the cervical region in Male mouse induced with MNU.**



245

246 Fig.17.Histological section of the above tumor showing necrosis (H&E X 40 obj.)

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248 3.3 Hematological Analysis

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250 The hematological parameters that were considered in this study are shown in Table 1. The
251 analysis shows that there were no significant differences ($P = 0.05$) amongst all the
252 parameters considered in this study between the control and induced mice. Generally,
253 eosinophils showed higher values in females (0.75 ± 0.35 ; 0.75 ± 0.35) than males ($0.5 \pm$
254 0.71 ; 0.5 ± 0.0), although levels within the induced and control in both sexes respectively
255 were the same (Table 1). **White blood cell count, lymphocytes and plasma levels were**
256 **not significantly different at $p = 0.05$ in both induced and control mice (both sexes).**
257 **Similarly, packed cell volume, hemoglobin content, red blood cells, neutrophils and**
258 **monocytes showed no significant differences at $p = 0.05$ (Table 1).**

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268 **Table 1: Hematological Parameters of MNU induced and control Albino Mice**

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Test samples	PCV (%)	Hb (g/dl)	RBC (L)	WBC X10 ³ (μL)	Platelets X10 ⁴ (μL)	Lymphocytes	Neutrophils	Monocytes	Eosinophils	Plasma
Fc	32	10.35	5.32	14.93	25.35	67	30.5	2	0.5	7.9
	39.5	12.95	6.4	5.95	11.6	67	29	3	1	7.5
Means±SD	35.75±5.3	11.65±1.84	5.86±0.76	10.44±6.35	18.48±9.72	67±0	29.75±1.06	2.5±0.71	0.75±0.35	7.7±0.28
F _i	37	12.15	6.15	13.25	23.05	60	39	1	0.5	6.75
	39	12.80	6.35	4.43	8.7	68	29	2	1	7.55
Means±SD	38±1.41	12.48±0.46	6.25±0.14	8.84±6.24	15.88±10.3	63.75±6	34±7.07	1.5±0.71	0.75±0.35	7.15±0.57
Mc	31	10.05	4.89	7.75	13.8	67	31.5	0.5	1	7.7
	38	12.60	6.4	7.4	9.2	70	29	1	0	7.5
Means±SD	34.5±4.95	11.33±1.8	5.65±1.07	7.58±0.25	11.5±325.3	68.5±2.12	30.25±1.77	0.75±0.35	0.5±0.71	7.6±0.14
M _i	31	10.15	5.21	11.38	15.9	67	32	0.5	0.5	7.6
	39	13	6.35	2.98	6.3	69	28	2.5	0.5	7.5
Means±SD	35±5.66	11.56±2.00	5.78±0.81	7.18±187.8	11.1±2146.6	68±1.41	30±2.83	1.5±1.41	0.5±0.0	7.55±0.07

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273 *F_c*-Female control; *F_i*-Female induced; *Mc*-Male control and *M_i*- Male induced; *PCV*- Packed Cell Volume; *Hb*- Hemoglobin; *RBC* - Red Blood Cell and *WBC*- White Blood
 274 Cell. (n=2)

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4. DISCUSSION

MNU is a very potent and reliable carcinogen with a severe toxicity in terms of the duration taken to induce cancer and bring about effects in organs of organisms, when compared to other known carcinogens (Franchi *et al.*, 2003). **It was not clear why the male induced mice presented a slightly higher mean body weight when compared to their male control counterparts, although these differences were not significant at $p = 0.05$. However, though rarely observed, similar findings occur in literature. Swiss Albino mice inoculated with Ehrlich Ascites Carcinoma had a maximum body weight gain of 20% compared to Cisplatin treated controls. No reasons were however given for this observation by Sathistha *et al.*, (2008). However it may probably indicate some degree of resistance by this strain of mice to the effects of the carcinogen induction.** When organisms are exposed to toxicants, their organs tend to accumulate such toxicants (Pelgrom *et al.*, 1995). The organism may metabolize the toxicant and excrete it. However, it is the accumulation of the toxicant in the organs of the induced mice, such as the liver, spleen and heart amongst others that is responsible for the abnormalities observed in these visceral organs as shown in this study.

The thymus is a specialized organ of the immune system. The only known function is the production and “education of T-lymphocytes (T cells) which are critical cells of the adaptive immune system. It is derived from the pharyngeal pouch and in relationship with other lymphatic organs takes a central super ordinate place (Franchi *et al.*, 2003). Induction with MNU in P53+/- mice resulted in the effacement of the thymic cortico-medullary architecture by diffuse sheets of lymphoblasts with large euchromatic nuclei, moderate to high number of mitotic figures, infiltration of lymphoblasts through the thymic capsule and presence of cluster or sheets of lymphoblasts in the spleen, liver and other visceral organs (Morton *et al.*, 2008). Increased lymphocytic activity was observed in the thymus of the female induced and the spleen of male induced mice respectively. Morton *et al.*, (2008) also observed enlarged nuclei sizes in the thymus of the induced mice. Both studies also reported the presence of thymic lymphomas in the strains used. Enlarged nuclei sizes and other abnormalities such as pleomorphic nuclei were also observed in the thymus in this study. These cellular abnormalities may therefore suggest a gradual progression towards the distortion reported by the above authors.

The heart and spleen of the induced mice also showed a number of abnormal features; an indication of distortion and degeneration as a result of exposure to the carcinogen. Although, reports of the effects of injected MNU on the histology of the heart seem very limited, some of the distortions have however been recorded by Palazzi and Kergozien-Framery (2009). These authors also reported other degenerations in the heart which included myofibrosis, distortion of muscle fibres and mineralization. Minardi and Maltoni (1998) reported splenic hemosiderosis, epitheliomorphous peritoneal mesothelioma and biphasic (solid epithelial and fusocellular pattern) as features of abnormalities in the spleen of an MNU induced rat. However, there were no histopathological changes recorded in the heart and spleen of rats exposed to MNU intravenously in a six month study conducted by Murata *et al.*, (2006).

The liver is an important organ of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites and is especially vulnerable to damage. Its activeness makes it more susceptible to carcinogen intoxication (Fazeli *et al.*, 2006). It is the primary target for carcinogen effect of more than two hundred chemicals (including pesticides, food additives, pharmaceuticals and industrial intermediates) tested in long-term toxicity safety assessment assays (Ward *et al.*, 2005). Most tumor initiating agents either generate or are metabolically converted to electrophilic reactants which bind covalently to cellular DNA (Slaga *et al.*, 1987; DiGiovannio, 1992). Free radicals and these modified DNA bases have been strongly implicated in carcinogenesis in general (Floyd, 1990; Molins, 1993). A high correlation has been found to occur between the dose of administered carcinogens such as

MNU and the levels of total DNA adducts in the liver. The liver is hypothesized to have a greater capacity for metabolizing toxicants (Izzotti *et al.*, 1999). MNU is a well known liver carcinogen which induces multiple cellular, molecular, and biochemical changes (Samaras *et al.*, 2010).

This study showed that MNU induction resulted in extensive degenerative changes in the liver varying from necrosis, multinucleation and increased nucleo-cytoplasmic ratio, cytoplasmic degeneration and pallor (Figures 11 & 12). Hyperplasia of the hepatic cells, congestion of central vein, and dilation of sinusoids were also observed in most of the histological sections of the induced mice. Similar observations have been reported by Shashi and Thapar (2000) in albino rabbits. The results of the histopathology of the liver were very consistent with early reports by Seigo *et al.*, (2002) in F344/DuCrj rats and Crj: BDF1 mice; and Brzoska *et al.*, (2003) in male Wistar rats. In rabbits, Wang *et al.*, (1993), also reported necrotic areas in the liver cells associated with vacuolar changes and proliferation of connective tissue fibres. Some of these observations have also been reported in the liver of rats exposed to formaldehyde (Bandman, 1994; and Tenq *et al.*, 2001). The increased sizes of the nuclei noticeable in both groups can be attributed to the increased activity going on in the cells since the liver is a very active metabolizing organ.

MNU exposure to the small intestine of albino mice caused an ulcerating effect as the walls of the small intestine (epithelial) were indented and eroded. Other morphological features noticed were nuclear pleomorphism, cytoplasmic pallor and vacuolization. Although there is paucity of reports showing the effects of injected MNU on the small intestine of albino mice, the morphological changes observed did not differ significantly from the abnormalities found in the other organs examined. Yoshizawa *et al.*, (2000) observed vacuolation in the posterior cortex of the lens of neo-natal Sprague Dawley rats, as cataract development progressed. **One male mouse was suspected to have developed a tumor in the cervical region at 60 days post induction. The tumor appeared to be encapsulated within a thin sheet of fibrous connective tissue. Further studies are however required to confirm the tumor and its origin. The histo-pathological examination of the tumor showed complete necrosis.** This result is very similar to the findings of several authors on tumors observed in different strains of rats and rabbits. (Shashi and Thapar, 2000 on albino rabbit; Brzoska *et al.*, 2003 and Franchi *et al.*, 2003 on Wistar rats and Morton *et al.*, 2008 on P53+/- mice)..

In comparison with other studies, Rivera *et al.*, (1994) administered MNU to female Sprague Dawley rats at a dosage of 50 mg/kg in animals of age 50, 80 and 110 days old. About 90% developed mammary tumors. Moon *et al.*, (1992) administered a single intravenous dose of 50 mg/kg in 120 day old Sprague Dawley rats. Their results showed a cancer incidence of 75-95% which were mainly mammary carcinomas. Franchi *et al.*, (2003) varied the dosage of MNU (intra-peritoneal) between 80-240 mg/kg in eight week old Wistar rats and found thymic lymphomas and splenic hemangiomas in the intermediate to high dose category (160-240 mg/kg). Morton *et al.*, (2008) also obtained thymic lymphomas in P53+/- mice. MNU resulted in cataract formation in 0-20 day old Sprague Dawley rats 7-30 days after exposure to a single intraperitoneal injection of 100 mg/kg (Yoshizawa *et al.*, 2000). In contrast, our study on MNU induction in albino mice using a single intra-peritoneal dose of 50 mg/kg showed that percentage tumor induction was minimal at the specified dose. This suggests that higher doses and/or a longer time frame may be required for carcinomas to develop in this strain of mice. It may also indicate that this strain is less susceptible than other strains such as Sprague Dawley and Wistar rats and may also likely explain the frequent use of the above strains in carcinogenesis models.

There were no significant differences in hematological parameters between the control and induced mice ($p = 0.05$). These results were then compared with the normal range of values expected in mice as stated by the Research Animal Resources of the University of Minnesota and the Iowa State University (Retrieved 17th February, 2012 from

www.ahc.umn.edu/rar/refvalues.html and www.lar.iastate.edu/index.php?option=com_content&). The normal range for hematological parameters in mice as given by the above were 10.2 – 16.6 g/dl for hemoglobin, white blood cell count (6-15 x 1000), lymphocytes, (55-95%), monocytes (1-4), platelets (160-410 x 1000) and eosinophils (0-4). Normal ranges for PCV, neutrophils and plasma were however not stated. Except for platelets in both male induced and control, and eosinophils in male controls, the range of hematological values observed in the control and induced animals of both sexes were mainly within the range of normal values stated above. However it is also pertinent to note that the ranges given above are not firm boundaries as age, sex, strain, sampling techniques and methodology may account for some degree of variation www.ahc.umn.edu/rar/refvalues.html.

The results of the hematological analysis were then compared with studies by other authors (Chakraborty et al, 2010; Raman et al, 2010) who observed significant differences in some hematological parameters between control and tumor bearing mice. Hemoglobin for instance, in the studies by Raman *et al*, (2010) had values of 12.92 ± 0.15 in control mice compared to 5.78 ± 0.4 g/dl in tumor bearing mice. Lymphocytes were $66.4 \pm 1.36\%$ in control compared to tumor bearing mice with $26.2 \pm 1.16\%$, while PCV values in both control and tumor bearing mice were much lower than obtained in this study at 16.86 and 25.98%. Values for PCV in studies by Akanni et al, (2010) at 24% and 31% for tumor bearing and control mice respectively were also lower than obtained in our study. Studies by Chakraborty et al, (2010) showed a similar pattern as that of Raman *et al*, (2010). Lymphocytes in normal unexposed mice were 67.13 ± 1.41 compared to tumor bearing mice at $18.54 \pm 0.28\%$, while red blood cell count in control and tumor bearing mice in studies of Sathistha et al, (2008) were 5.03 ± 0.28 and 2.14 ± 0.34 respectively.

The disparity between the hematological results in this study and that presented by the above authors may therefore be due to the fact that these authors examined tumor-bearing rats; while in this study, only one of the induced mice had advanced to the tumor-bearing stage (figures 16 and 17) during the study period. It is hypothesized that the duration of this study was probably not sufficient to have resulted in significant differences among the hematological parameters between the induced and the control animals when compared with some previous studies. The fact that the hematological values were mainly within normal range in both induced and controls' further supports this assertion. It is also illustrative of the challenges in human cancer diagnosis and treatment, in which symptoms often appear when the disease is at an advanced stage, thus making treatment of the disease challenging.

4. CONCLUSION

Reports abound on MNU induction in Sprague Dawley rats, less commonly in Hamsters, and Wistar rats. Our studies provide information on MNU induction in albino mice, which is illustrative of differences in the susceptibility of various strains to the carcinogen induction. Percentage tumor induction was minimal at a dose of 50 mg/kg, with only one mouse suspected to have developed a tumor in the cervical region at about 60 days post induction. No acute toxicity was also observed except for the mouse with tumor.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Author A initiated the study and provided funds via the research grant. Author B managed the experimental, draft writing, and corrections of the manuscript, Author C carried out the experimental work and prepared first draft, Author D carried out the histology, while Author E conceptualized and supervised the study. All authors read and approved the final manuscript."

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

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