

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

ABSTRACT

Chemically induced carcinogenesis models in rats and mice are widely used for studying the biology of cancer and for developing and evaluating cancer prevention strategies. This study was performed to assess the effects of N-methyl-N-nitrosourea (MNU) on haematology, and the histology of selected organs in albino mice. Eighty male and female mice eight-week old, were subdivided into four groups of twenty each. Forty male and female mice received 50mg/kg of MNU in 1mL of 0.9% sodium chloride via intra-peritoneal injection. MNU induction impacted different morphological abnormalities in induced mice: debris formation (heart), vacuolization (thymus, small intestine), and multi-nucleation (spleen, liver), cytoplasmic pallor (heart, liver, small intestine), vesicular nuclei (liver), necrosis (heart, liver), haemorrhage (thymus) and destruction of cellular epithelia (small intestine). Increased nuclear-cytoplasmic ratio, enlarged nuclei sizes and cytoplasmic degeneration were also common features of all organs examined. One of the induced mice developed a neck tumour eight weeks after induction. The histological section of the tumour showed complete necrosis. Except for increased nuclei sizes in the liver, these abnormalities were not observed in control mice. Haematological analysis showed an increase in packed cell volume, haemoglobin, red blood cell count and monocytes of the induced mice, although these differences were not significant ($p < 0.05$) between the induced and control. Exposure to MNU at 50mg/kg over a 28-week period caused varying degrees of abnormalities in some organs of induced mice. The duration of the study however appeared insufficient for the development of any pronounced haematological effects in the induced mice.

Keywords: *Carcinogenesis, Histopathology, Haematology, N-methyl-N-nitrosourea, Albino mice, Tumors.*

1.0. INTRODUCTION

Cancer is a class of diseases in which a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via the lymph or blood). These three malignant properties of cancers differentiate them from benign tumours, which are self-limiting and do not invade or metastasize. Most cancers form a tumour but some, like leukemia, do not (Cancer Research, UK, 2007). Cancer is usually treated by chemotherapy, radiotherapy and surgery. The focus of current research in chemotherapy is to develop target candidates for drugs which have better cyto-toxicity and less side effects than

the platinum based cisplatin, which is presently widely used in cancer treatment. Research is on to find possible drug targets with comparable toxicity and less side effects.

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 tumour 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 tumours on varying strains of rats and mice.

Its ability to induce cataractogenesis in Sprague-Dawley rats and its establishment of abnormality to lymph nodes, spleen, liver, lung, kidney, bone marrow, eye, and other visceral organs have been reported in these rats. Grubbs *et al.*, (1981) examined its effects on hamster rats. 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 Wister rats while Seigo *et al.*, (2002) studied the effects of this carcinogen on F344/DUCRJ rats and Crj: BDF1 mice. Morton *et al.*, (2008) investigated its effects on P53+/- mice, while its effects on the haematology of Wister rats were reported by Akanni *et al.*, (2010). However, despite the enormous literatures that abound in the use of this carcinogen to induce varying carcinomas in different strains of rats and mice, there appears to be paucity of information as regards its effects in swiss albino mice. Differences in susceptibility may likely occur across the different strains, which can give an indication of genetic variation in response (Satoru *et al.*, 1985; Festing, 2010). Information on tumor induction in albino mice by the carcinogen MNU would therefore prove valuable in this regard. Furthermore, reports on the haematology of tumour bearing rats particularly albino mice appear to be scarce.

The use of a single intra-peritoneal dose of MNU induce carcinogenesis in mice has been established by Morton *et al.*, (2008). This study is the first of a two part study aimed at carrying out a histological evaluation of selected organs of the circulatory (heart), lymphoid (thymus, spleen) and the digestive systems (liver, and small intestine) in MNU injected Swiss albino mice. This is to provide information on the effects of this carcinogen in this less studied strain as well as to provide added information on the effects of the MNU on some haematological parameters [Packed Cell Volume (PCV), Haemoglobin (Hg), Red Blood Cell (RBC), White Blood Cell (WBC) and its differentials (Lymphocytes, Neutrophils, Monocytes and Eosins), Platelets and Plasma] in the mice. The second part (currently in progress) is designed to evaluate the in-vivo effects of some synthesized copper complexes on the tumor treated mice.

2.0. MATERIALS AND METHODS

2.1 Experimental Animals.

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

2.2 Chemical Carcinogen.

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

2.2.1 Preparation Mode for Carcinogen

50mg/kg doses of MNU were given to the mice. Based on the average weight of 24.47g and 20.14g for males and females respectively, the mice were thus given a calculated dosage of approximately 0.1ml MNU for both the males and females. The MNU was dissolved in 1ml of 0.9 % sodium chloride and 10mg of MNU was dissolved in the 1ml of 0.9% of the sodium chloride.

2.3 Experimental design

2.3.1 Induction with the carcinogen MNU

Mice of both sexes were randomly divided into four groups (M_I , F_I , M_C and F_C). Groups M_C and F_C served as the control (members of this group were not induced) while groups M_I and F_I received 0.1ml of MNU, via intraperitoneal injection. After the carcinogen induction, the mice were kept on basal diet and water *ad libitum* and were subsequently weighed weekly to monitor changes in their weight. From the second week after induction, animals were palpated twice weekly to monitor possible tumour appearance. Complete gross examination was carried out for detection of tumour. One mouse from each group was sacrificed at the end of every four weeks. The mice were anesthetized with chlorofoam 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.

2.3.2 Collection of Blood Samples and Measurement of Haematological Parameters

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 Haemoglobin (Hb) content of the blood obtained from the mice were analyzed at the haematology 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.

Mean and standard deviations of the haematological indices and the relative weights in each group were calculated and the differences between group means determined using the student T-test from Microsoft Excel, 2007. Differences were significant at $P < 0.05$.

3.0. RESULTS

3.1 Body Weights and Clinical Signs

Members of both groups gained body weight progressively during the experiment (Figure 1), with the induced males and control males reaching a maximum weight of 40.03g and

40.37g respectively, and at the expiration of the study period (28th week), their weights did not differ significantly ($P < 0.05$) from the control. However, the female control presented a mean body weight ($\pm$ SD) - (28.48g $\pm$ 0.95) higher than that of the female induced (27.24g $\pm$ 0.73). The average weight of induced male mice was more (0.15g) in weight than the male control mice. The induction with MNU appears not to have caused any significant changes in the behaviour of both the control and the induced mice. Majority of the induced mice were healthy and show no observable differences from the control. The mice also did not show any form of laboured or rapid breathing patterns. Tumour of the neck was observed in one of the induced mice barely eight weeks after the carcinogen induction. Except for the mouse with tumor, no mortality was observed in the induced or control mice.

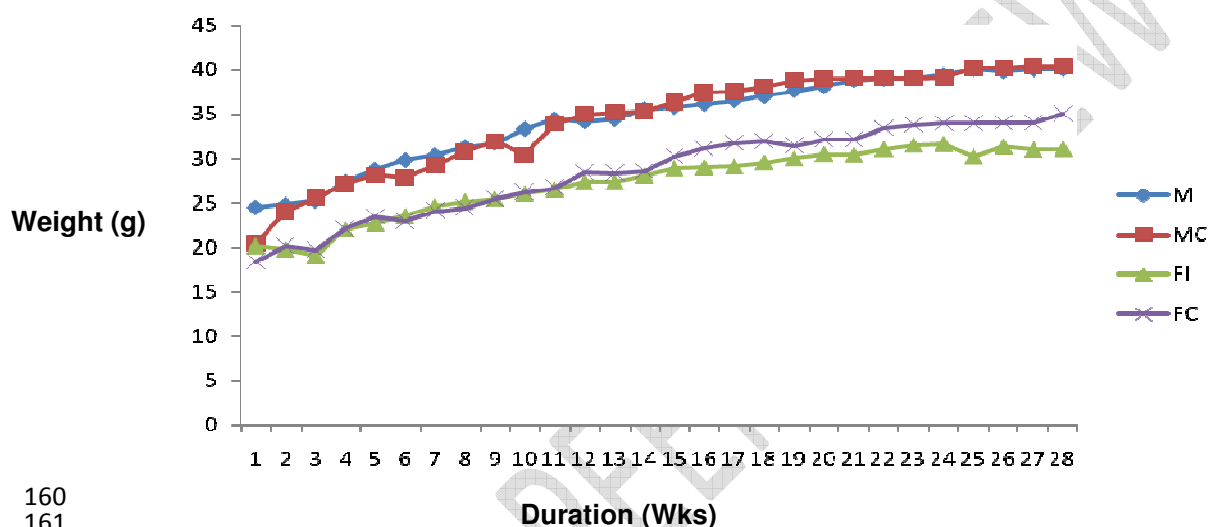


Fig. 1: The average weight of both the induced and control albino mice over a 28-week study period.

3.2 Histopathology.

There were varying degrees of lesions and abnormalities in the exposed animals when compared to the control. While Figure 2 shows the histological section of the thymus of a control mouse with normal architecture and well delineated cells; prominent vacuoles with lymphocytes infiltration (Figure 3), cytoplasmic degeneration and prominent vacuole formation infiltrated with numerous lymphocytes (Figure 4) were some of the abnormalities observed in the thymus of the exposed mice. Figure 5 shows haemorrhage, nuclear pleomorphism, cytoplasmic degeneration and increased nuclear-cytoplasmic ratio in the thymus of induced mice. While normal architecture with well delineated striated muscles was observed in the heart of the control mice (Figure 6), the morphological changes observed in

the heart of the induced mice included nuclear pleomorphism, debris formation along with cytoplasmic pallor, and necrosis (Figures 7, 8, and 9).

The spleen of the control showed normal architecture, while some of the abnormalities observed in the induced mice were: multinucleation (Figure 11), cytoplasmic degeneration, presence of lymphocytes, nuclear pleomorphism (Figure 12), as well as increased nucleocytoplasmic ratio (figure 13). The liver of the MNU treated mice (Figures 15-18) were also inundated with majority of the aforementioned abnormalities in other organs. Abnormalities observed in the liver of the induced mice include necrosis (Figure 15), multinucleation (Figures 16 and 18), increased nucleocytoplasmic ratio (Figure 16), cytoplasmic degeneration associated with cytoplasmic pallor (Figure 17), vesicular nucleus and nuclear pleomorphism (figure 18). There were also dilation of the sinusoids, and disintegration of hepatic cells including necrosis of hepatocytes, and enlarged nuclei in the liver of the treated mice.

Other observable features were; serious disarrangement and degeneration / distortion of the regular hexagonal shape of the hepatocytes. Furthermore, most of the cytoplasm have lost their eosinophilic colours, the radial architecture of the cells has also been altered and the cells thus lack distinct outline and appear reduced in size indicating distortion of the hepatic architecture. Some of these features were however, observed in the histological sections of the control mice (Figure 14). The histological sections of the small intestine of the control mice showed well defined architecture with unaltered epithelial (Figure 19). However, cytoplasmic pallor and degeneration of the epithelial of the small intestine were noticed as illustrated in Figures 21 and 22. The histological section of the tumour of the neck observed in one of the exposed mice barely eight weeks after exposure showed complete necrosis (Figure 24).

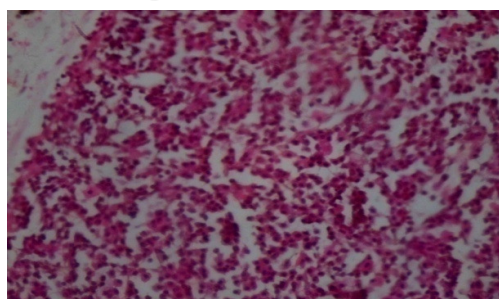


Fig.2. Control thymus female showing normal architecture. (H & E X 40).

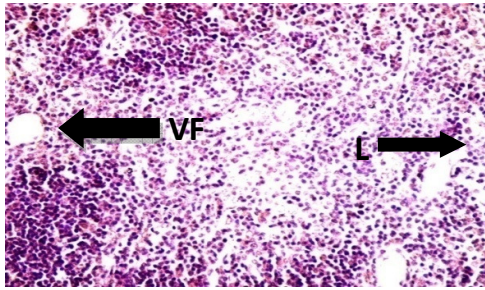


Fig.3.Induced Thymus Male showing Vacuole Formation (VF) and Presence of numerous Lymphocytes (L) (H&E X 40 obj.)

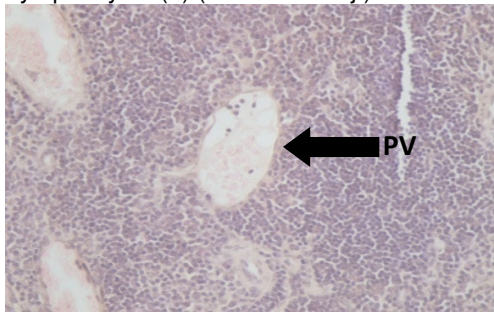


Fig.4.Induced Thymus Male showing Degeneration of Cytoplasm, Prominent Vacuoles (PV) formation infiltrated with lymphocytes. (H&E X 40 obj.)

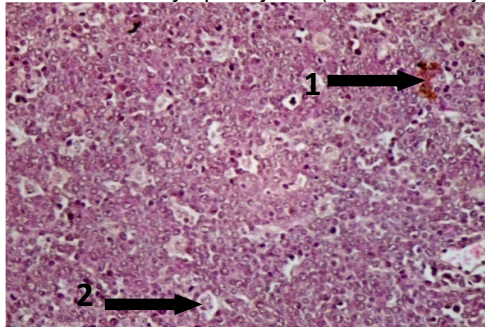


Fig.5.Induced Thymus Male showing (1). Haemorrhage, (2.) Nuclear Pleomorphism. Figure also shows cytoplasmic degeneration (CD) and Increased Nuclear- Cytoplasmic ratio

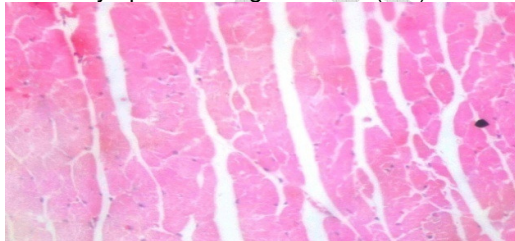


Fig.6.Heart Control Male showing normal architecture with well delineated striated muscle and evenly distributed cardiomyocytes (H&E X40 obj.)

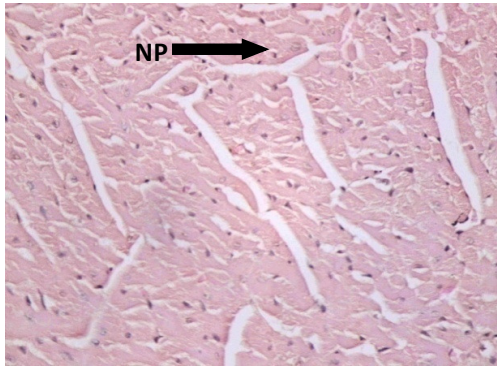


Fig.7.Heart Induced Male Showing Nuclear Pleomorphism (NP) (H&E X40 obj.)

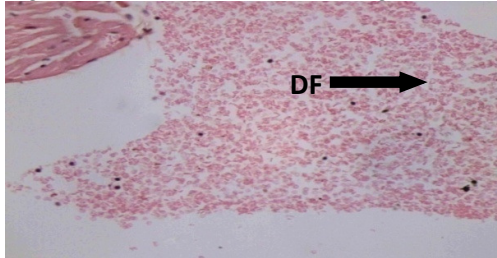


Fig.8.Heart Induced showing Debris Formation (DF) along with Cytoplasmic pallor (H&E X40 obj.)

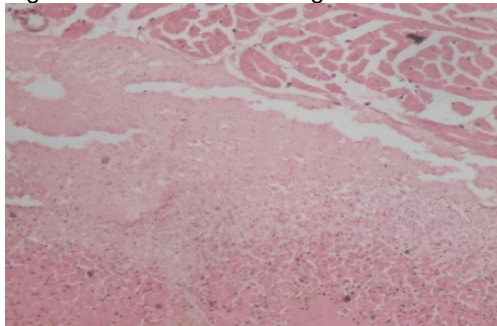


Fig.9.Heart Induced showing Necrosis (H&E X40 obj.)

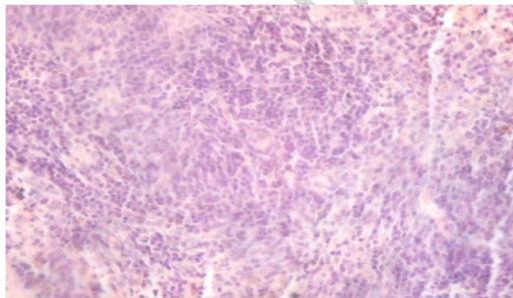


Fig.10.Spleen Control showing normal architecture (H&E X 40 obj.)

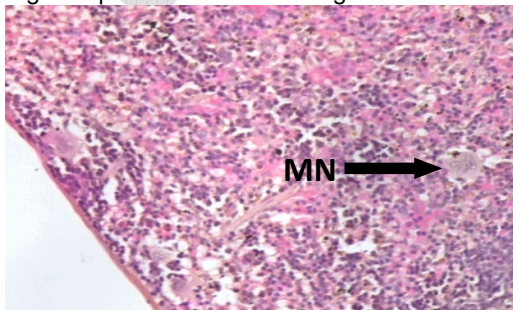


Fig.11.Spleen Induced showing Multinucleation (MN) of the cell (H&E X40 obj.).

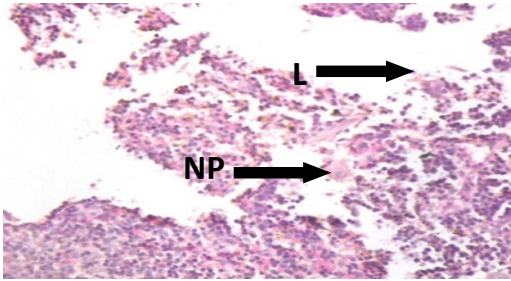


Fig.12. Induced Spleen Male showing Cytoplasmic Degeneration(CD), presence of numerous Lymphocytes (L) and Nuclear-Pleomorphism (NP). H&E (X40 obj.)

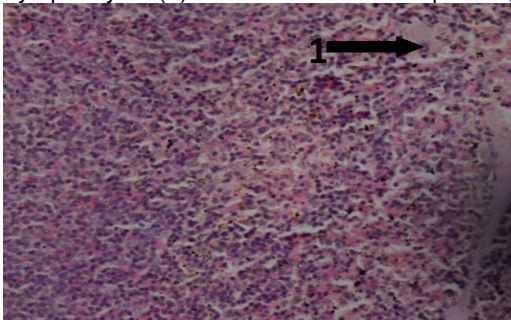


Fig.13. Spleen Induced Male showing (1) increased Nucleo-cytoplasmic ratio (H&E X40 obj.).

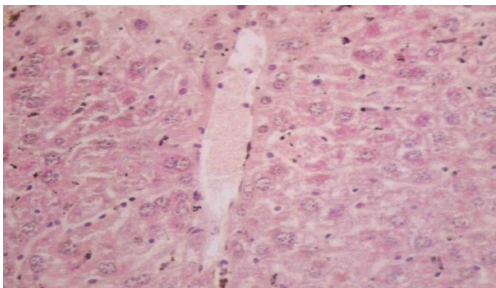


Fig.14.Histological section showing normal appearance of mice liver (H&E X 40 obj.)

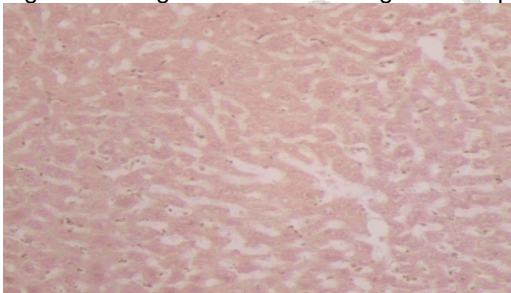


Fig.15.Histological section of the liver of an induced mice showing necrosis (H&E X 40 obj.)

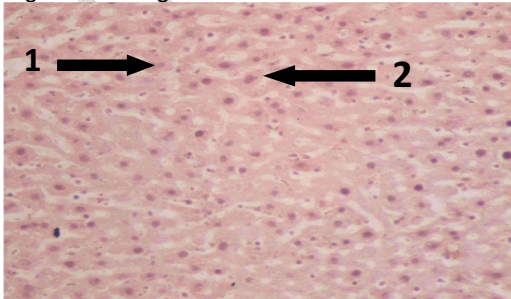


Fig.16.Histological section of the liver of an induced mice showing (1) multinucleation and (2) increased nucleocytoplasmic ratio

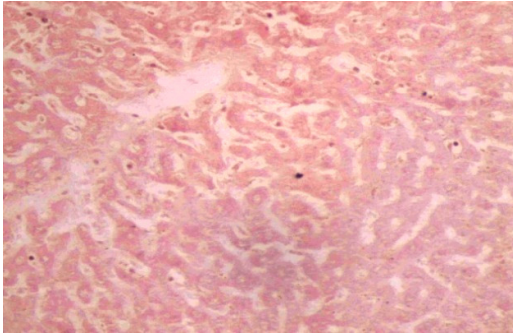


Fig.17.Histological section of an induced liver showing cytoplasmic degeneration with cytoplasmic pallor (H&E X40 obj.)

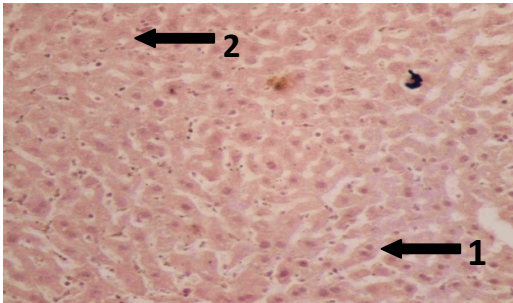


Fig.18.Histological section showing induced liver with (1) multinucleation (2) vesicular nucleus. The slide also show nuclear Pleomorphism (H&E X 40 obj.)

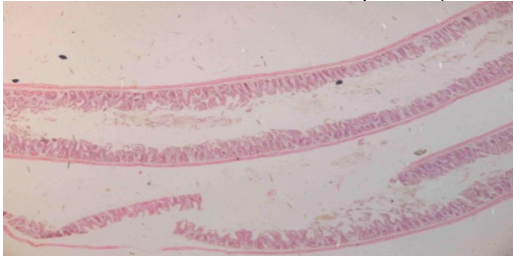


Fig.19.Histological section of the small intestine of control mice showing normal architecture and unaltered epithelial (H&E X 40 obj.)

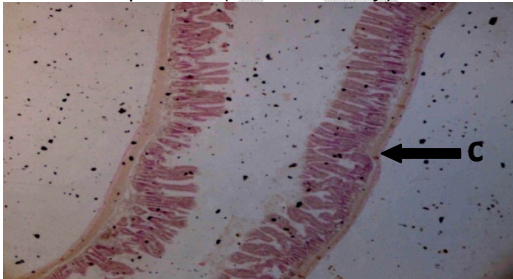
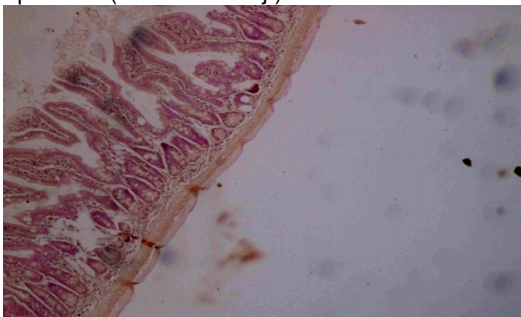
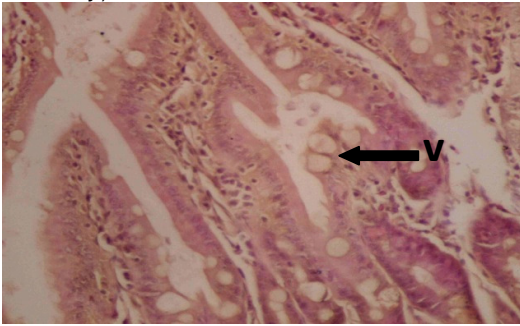


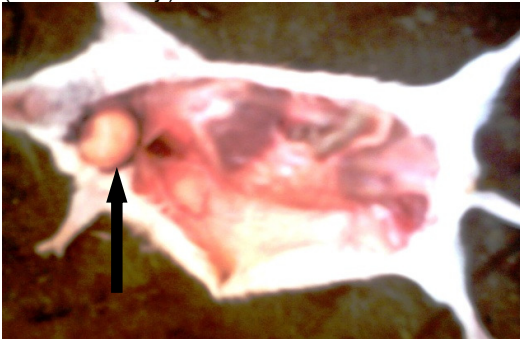
Fig.20.Histological section of the small intestine of an exposed animal showing 'C' corrosion of the epithelial (H&E X 40 obj.)



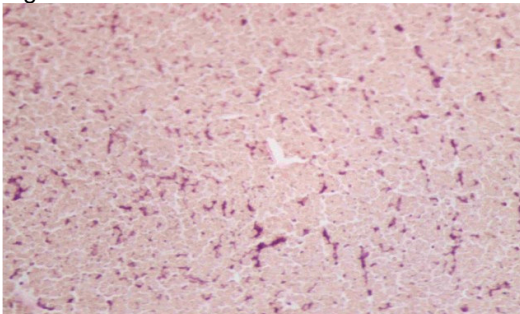
277 Fig.21.Histological section of the small intestine of the induced mice showing cytoplasmic pallor (H&E
278 X 10 obj.)



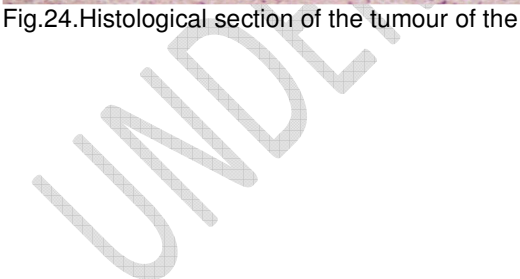
279 Fig.22.Section of the small intestine of an induced mice showing presence of numerous vacuoles
280 (H&E X 40 obj.)
281



282 Fig.23.Tumour of the neck in mouse induced with MNU
283



284 Fig.24.Histological section of the tumour of the neck showing necrosis (H&E X 40 obj.)
285



286 **Table 1: Haematological Parameters of MNU induced and control Albino Mice**

287

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	14925	25.35	67	30.5	2	0.5	7.9
	39.5	12.95	6.4	5950	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	13250	23.05	60	39	1	0.5	6.75
	39	12.80	6.35	4425	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

288

289

290

291 Fc-Female control; F_i- Female induced; Mc-Male control and M_i- Male induced; PCV- Packed Cell Volume; Hb- Haemoglobin; RBC - Red Blood Cell and

292 WBC- White Blood Cell.

294 The haematological parameters that were considered in this study are shown in Table 1.
295 The analysis shows that there were no significant differences ($P < 0.05$) amongst all the
296 parameters considered in this study between the control and induced mice. Generally,
297 eosinophils showed higher values in females (0.75 ± 0.35 ; 0.75 ± 0.35) than males ($0.5 \pm$
298 0.71 ; 0.5 ± 0.0), although levels within the induced and control in both sexes respectively
299 were the same (Table 1). White blood cell count, platelets, lymphocytes and plasma levels
300 showed increased levels in control mice compared to the induced in both sexes (Table 1).
301 However, packed cell volume, haemoglobin content and red blood cell count were increased
302 in the induced mice compared to the control in both sexes (Table 1). Neutrophils and
303 monocytes were increased in induced females (34.7 ± 7.07 ; 1.5 ± 1.41) and induced males
304 compared to the control groups respectively (29.75 ± 1.06 ; 0.75 ± 0.35), but all these
305 differences were not significant at $P < 0.05$ (Table 1).

307 4.0. DISCUSSION

309 MNU is a potent and a very reliable carcinogen with a severe toxicity in terms of the
310 duration taken to induce cancer and to bring about effects in organs of organisms when
311 compared to other known carcinogens (Franchi *et al.*, 2003). When organs of organisms are
312 exposed to toxicants, these organs will accumulate such toxicants (Pelgrom *et al.*, 1995). The
313 organism can metabolize the toxicant and excrete it. However, it is the accumulation of the
314 toxicant in the organs like liver, spleen and heart amongst others of the exposed mice that is
315 responsible for the abnormalities observed in these visceral organs as seen in this study.

317 The ability of the thymus which is a specialized organ of the immune system to fight
318 infections also resulted in the thinning of the corticle and progressive loss by the appearance
319 of hyperplastic and neoplastic cells (Patrica *et al.*, 1990). Induction with MNU resulted in the
320 formation of hyperplasia and neoplasm in the thymus of induced mice in studies by Patrica *et al.*,
321 (1990). Although, these abnormalities were not observed in the thymus of the induced
322 mice, evidences from the observed abnormalities indicate that there is a progression towards
323 the distortion reported by Patrica *et al.*, (1990). The findings in this study however support
324 earlier reports by Morton *et al.*, (2008), where large nuclei sizes and infiltrations of
325 lymphocytes were some of the abnormal features observed in the thymus of the induced
326 mice.

327 The heart and spleen of the induced mice also showed many of the above mentioned
328 features; an indication of distortion and degeneration as a result of exposure to the
329 carcinogen. Although, reports of the effects of injected MNU on the histology of the heart
330 seem very limited, some of the distortions have however been recorded by Xavier and Sylvie
331 (2009). These authors also reported other degenerations in the heart which included
332 myofibrosis, distortion of muscle fibres and mineralization. Minardi and Maltoni (1998)
333 reported splenic hemosiderosis, epitheliomorphous peritoneal mesothelioma and biphasic
334 (solid epithelial and fusocellular pattern) as features of abnormalities in the spleen of an
335 MNU induced rat. However, there were no histopathological changes recorded in the heart
336 and spleen of rats exposed to MNU intravenously in a six month study (Murata *et al.*, 2006).

337 The liver is an important organ of metabolism, detoxification, storage and excretion of
338 xenobiotics and their metabolites and is especially vulnerable to damage. Its activeness
339 makes it more susceptible to carcinogen intoxication (Fazeli *et al.*, 2006). It is the primary
340 target for carcinogen effect of more than two hundred chemicals (including pesticides, food
341 additives, pharmaceuticals and industrial intermediates) tested in long-term toxicity safety

assessment assays (Ward *et al.*, 2005). Most tumour 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 like MNU and the levels of total DNA adducts in the liver. The liver is hypothesized to have a greater capacity for metabolizing toxicants (Izzolti *et al.*, 1999). The carcinogen MNU in specifics 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 multinucleation and increased nucleo-cytoplasmic ratio (Figure 16), cytoplasmic degeneration and pallor (Figure 17), vesicular nucleus and nuclear pleomorphism (Figure 18). 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 (2001) 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 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 Teng *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 causes 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 noticeable did not differ significantly from the abnormalities that have been observed and reported in some other organs like the liver, thymus, spleen and heart. The histopathological examination of the tumour in the neck observed in one of the exposed mice showed complete necrosis. This result is very similar to the findings of several authors on tumours 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).

There were no significant differences in haematological 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 haematological parameters in mice as given by the above were 10.2 – 16.6 g/dl for haemoglobin, 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 haematological 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 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 haematological analysis were then compared with studies by other authors (Chakraborty *et al*, 2010; Raman *et al*, 2010) who observed significant differences in some haematological parameters between control and tumour bearing mice. Haemoglobin 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 tumour bearing mice. Lymphocytes were $66.4 \pm 1.36\%$ in control compared to tumour bearing mice with $26.2 \pm 1.16\%$, while PCV values in both control and tumour 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 tumour 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 tumour bearing mice at $18.54 \pm 0.28\%$, while red blood cell count in control and tumour bearing mice in studies of Sathistha *et al*, (2008) were 5.03 ± 0.28 and 2.14 ± 0.34 respectively.

The disparity between the haematological results in this study and that presented by the above authors may therefore be due to the fact that these authors examined tumour-bearing rats; while in this study, only one of the induced mice had advanced to the tumour-bearing stage (figures 40 and 41) during the study period. It is hypothesised that the duration of this study was probably not sufficient to have resulted in significant differences among the haematological parameters between the induced and the control animals when compared with some previous studies. The fact that the haematological 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.

5. 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 tumour induction was minimal at a dose of 50 mg/kg, with only one mouse developing tumour at about 60 days post induction. No acute toxicity was also observed except for the mouse with tumour. Although tumour incidence was low at the specified dosage, the histo-pathological abnormalities observed indicate a gradual progression towards carcinogenesis.

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