British Journal of Applied Science & Technology
3(1): 17-33, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

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

A. A. Osowole[1], A. A. Oni[2*], F. O. Onadje[2], K. C. Onyegbula[3] and A. T. Hassan[2]

[1]Department of Chemistry, University of Ibadan, Ibadan. Nigeria.
[2]Department of Zoology, University of Ibadan, Ibadan. Nigeria.
[3]Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan. Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AAO initiated the study and provided funds via the research grant, Author AAOni managed the experimental, draft writing, and corrections of the manuscript, Author FOO carried out the experimental work and prepared first draft, Author KCO carried out the histology, while Author ATH conceptualized and supervised the study. All authors read and approved the final manuscript.

Research Article

Received 17th July 2012
Accepted 12th November 2012
Published 17th December 2012

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

**Corresponding author: E-mail: adeyoni2006@yahoo.co.uk;*

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 caused varying degrees of abnormalities over a 28-week period 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 [1]. 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. Its effects on hamsters have been examined [2]. The induction of mammary tumours in Sprague-Dawley rats has also been well reported [3,4,5,6]. Its effects on Wistar rats were examined by [7], while [8] reported its effects on the hematology of Wistar rats.

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 [9,10]. 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, [3,4,6]; thymic lymphomas were observed in Wistar rats and P53+/- mice. Splenic hemangiomas and small intestine adenomas were also reported respectively [7,11]. MNU was also found to induce cataracts in 0-20 day old Sprague Dawley rats [12]. 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 and mice, 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 [4,6,13]. This dosage is ideal for applications in which rapid tumor induction with high yield is desired [14]. 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.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Eighty (80) eight week 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 the carcinogen

A single dose of 50mg/kg of MNU was administered to the mice via intra-peritoneal injection. The carcinogen (10mg) was dissolved in 1ml of 0.9 % sodium chloride. 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.

2.3 Experimental Design

2.3.1 Induction with the carcinogen MNU

Mice of both sexes were randomly divided into four groups of twenty each (MI, FI, MC and FC). Groups MC (male control) and FC (female control) served as the control (members of this group were not induced nor given placebo) while groups MI, (male induced) and FI (female induced) received 0.1ml of MNU via intra-peritoneal 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 tumor appearance. Complete gross examination was carried out for detection of tumor. The animals were monitored for 28 weeks. One mouse from each of the four groups was sacrificed for histological examination at the end of every four weeks. This was to allow for sufficient sample size for the second phase of this study. Although less common, studies involving the use of one mouse per time point/group for histological evaluation in cancer studies do occur in literature [15,16]. In all, a total of twenty eight mice comprising seven mice from each of the four groups were sacrificed over the 28-week study period and processed for histological examination. In addition, a tumor bearing mouse which died before the end of the study period was also

processed for histological examination, bringing the total number of mice 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 heparinsed 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 adopted in references 17 and 18.

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 (Fig. 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 (± SD) of 28.48g ± 0.95, which was higher than the mean body weight of the female induced group (27.24g ± 0.73). However this difference was not significant at $p = 0.05$. Furthermore, the average weight of the induced male mice (34.38g ± 0.94) was higher than the male control mice (34.23g ± 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.

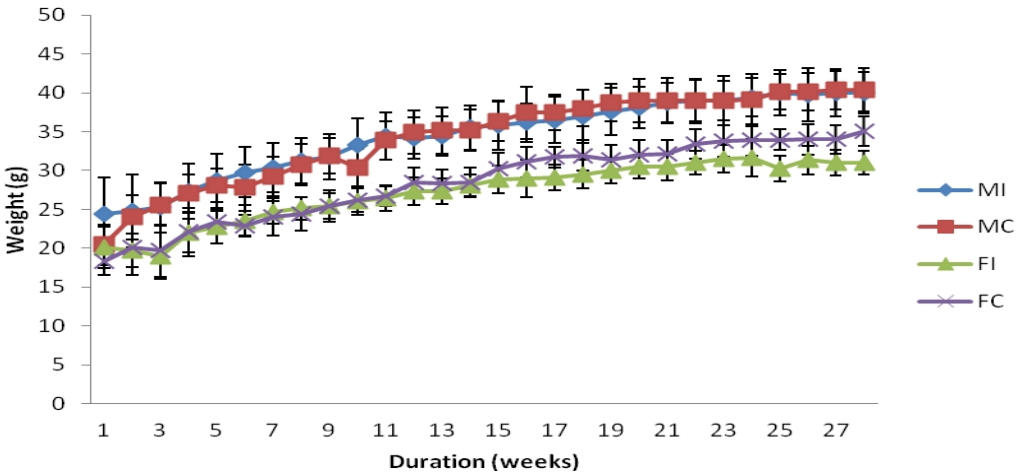

Fig. 1. The average weight of both the induced and control albino mice over a 28-week study period

Mean ± S.D = Mean values ± Standard deviation of twenty mice per group.
However there was variation in N over the study period due to the exclusion of animals sacrificed for histology at the end of every four weeks (seven in each group over the study period). One mouse also died in the group MI before the end of the study period.

3.2 Histopathology

There were varying degrees of lesions and abnormalities in the induced animals when compared to the control. Abnormalities were observed in all the seven induced male and female mice respectively, which were sacrificed over the study period. Except for increased nuclei sizes in the liver, all the control mice sacrificed (seven in each group) did not record any abnormalities or lesions. Representative figures are however used here for the comparison. The figures presented represent some of the abnormalities observed in the organs of different mice examined during the study period. Fig. 2 shows the histological section of the thymus of a female control mouse with normal architecture and well delineated cells. Vesicular nuclei, nuclear pleomorphism, increased nucleo-cytoplasmic ratio and increased lymphocytic activity (Fig. 3) were some abnormalities observed in the thymus of the induced mice. While normal architecture with well delineated striated muscles was observed in the heart of the control mice (Fig. 4), the morphological changes observed in the heart of the induced mice included necrosis and scanty lymphocyte infiltration (Fig. 5).

The spleen of the control showed normal architecture (Fig. 6), while some of the abnormalities observed in the induced mice were cytoplasmic degeneration, increased lymphocytic activity and nuclear pleomorphism (Fig. 7). The liver of the MNU treated mice showed abnormalities such as necrosis and cytoplasmic degeneration associated with cytoplasmic pallor (Fig. 9). 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.

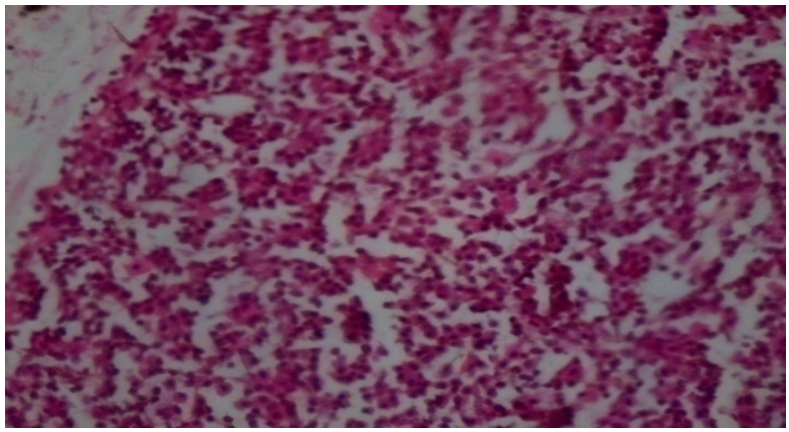

Fig. 2. Control Thymus Female showing normal architecture (Mag. x 400)

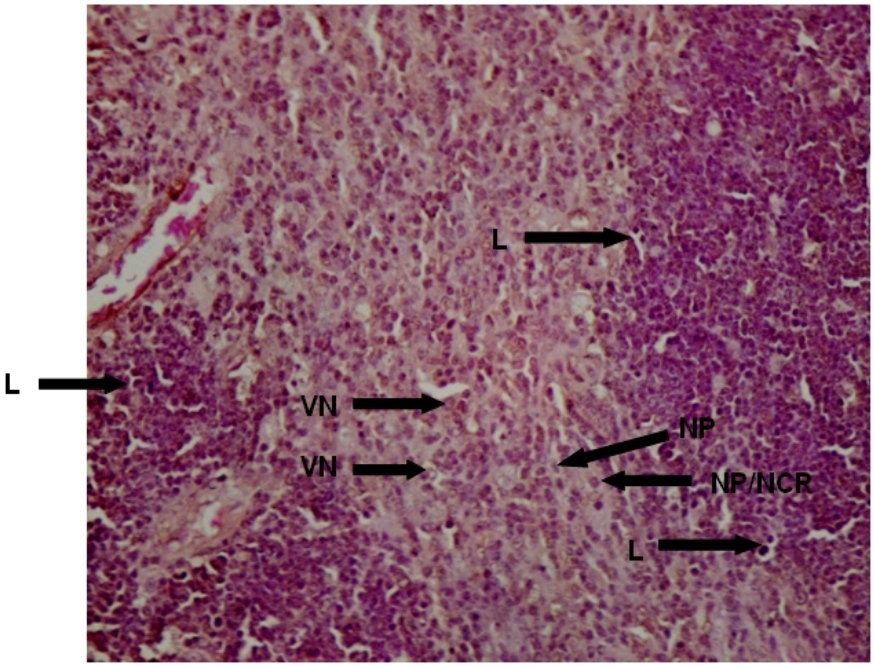

Fig. 3. Induced Thymus Female showing Vesicular Nuclei (VN), irregularly shaped nuclei or Nuclear Pleomorphism (NP), increased Nucleo-Cytoplasmic Ratio (NCR) and increased lymphocytic activity (L) (dark colored cells) (Mag. x 400)

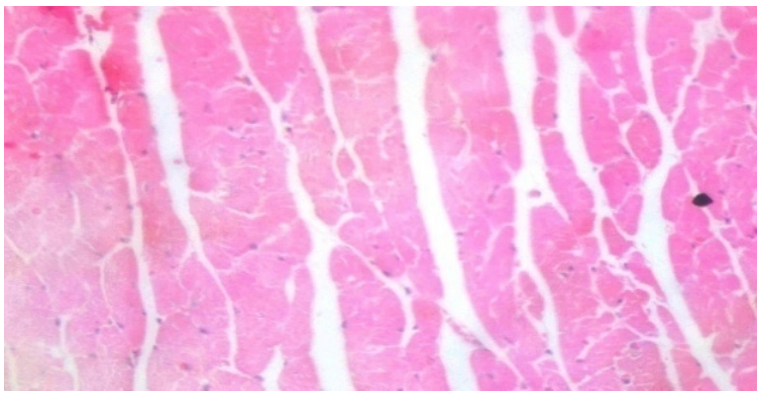

Fig. 4. Heart Control Male showing normal architecture with well delineated striated muscles and evenly distributed cardiomyocytes (Mag. x 400)

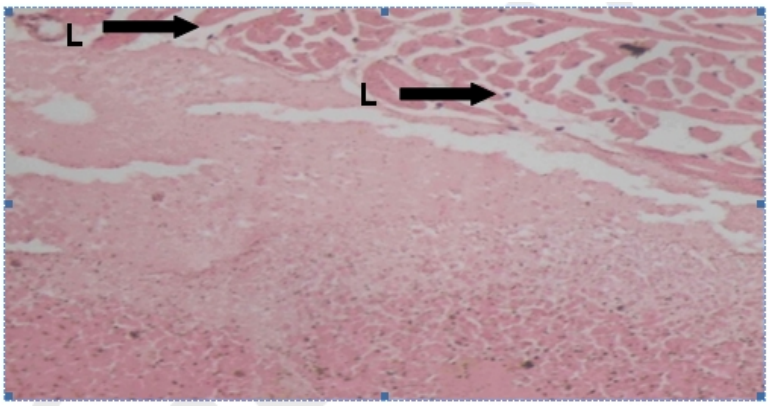

Fig. 5. Heart Induced Male Showing Necrosis and scanty lymphocytes (Mag. x 400)

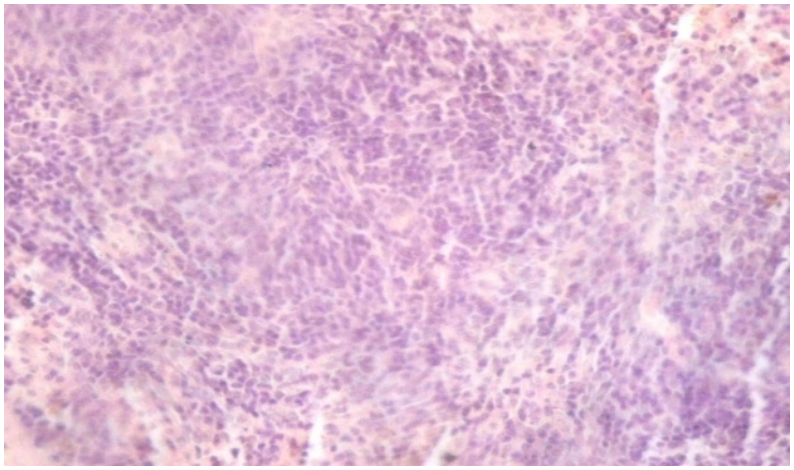

Fig. 6. Spleen Control Male showing normal architecture (Mag. x 400)

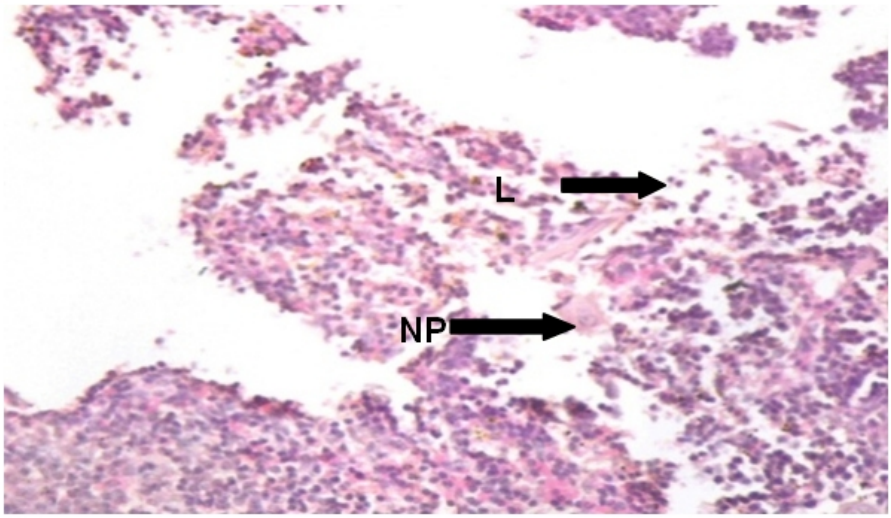

Fig. 7. Induced Spleen Male showing Cytoplasmic Degeneration (CD), increased Lymphocytic activity (L) and Nuclear-Pleomorphism (NP) (Mag. x 400).

Other observable features were; serious disarrangement and degeneration / distortion of the regular hexagonal shape of the hepatocytes. Furthermore, most of the cytoplasms 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 (Fig. 8). The histological sections of the small intestine of the control mice showed well defined architecture with unaltered intestinal wall (Fig. 10). However, degeneration of the intestinal wall of the small intestine was observed in the induced mice as illustrated in Fig. 11. The histological section of the cervical tumor observed in one of the exposed mice barely eight weeks after exposure showed complete necrosis (Fig. 13).

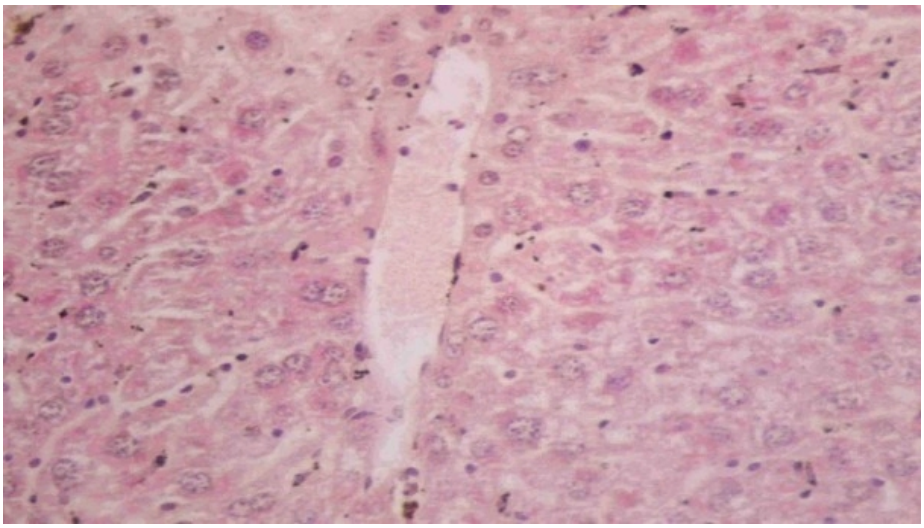

Fig. 8. Histological section showing normal appearance of female liver (Mag. x 400)

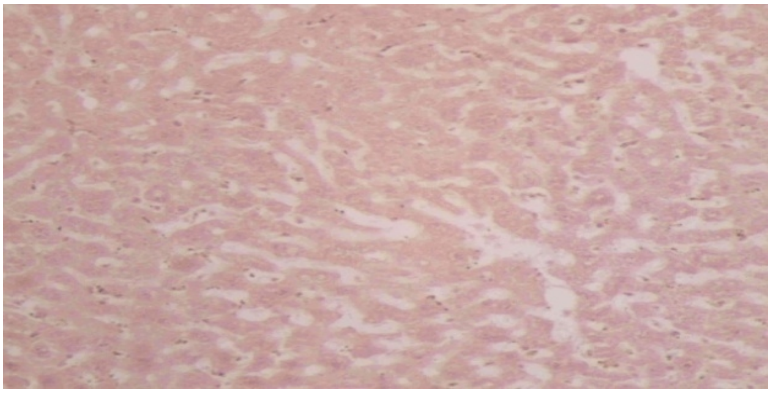

Fig. 9. Histological section of the liver of an induced female showing necrosis and cytoplasmic degeneration associated with cytoplasmic pallor (Mag. x 400).

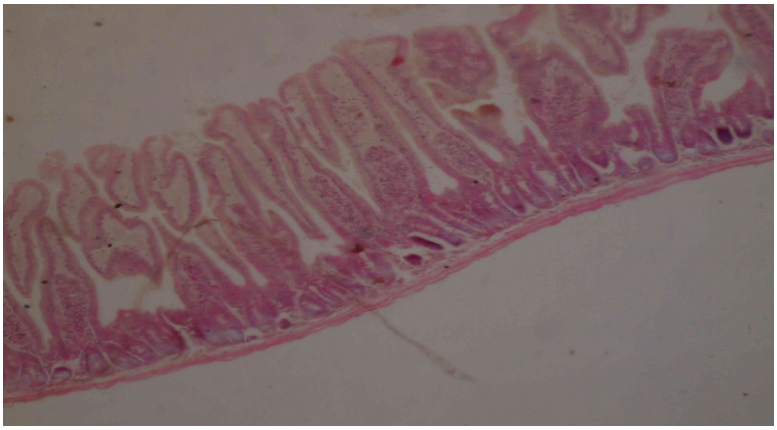

Fig.10. Histological section of the small intestine of male control mice showing normal architecture and unaltered intestinal wall (Mag. x 100).

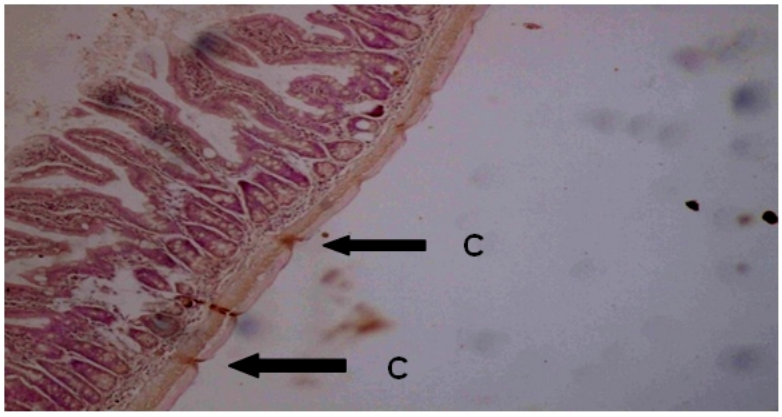

Fig. 11. Histological section of the small intestine of an induced male showing corrosion of the intestinal wall (C) (Mag. x 100)

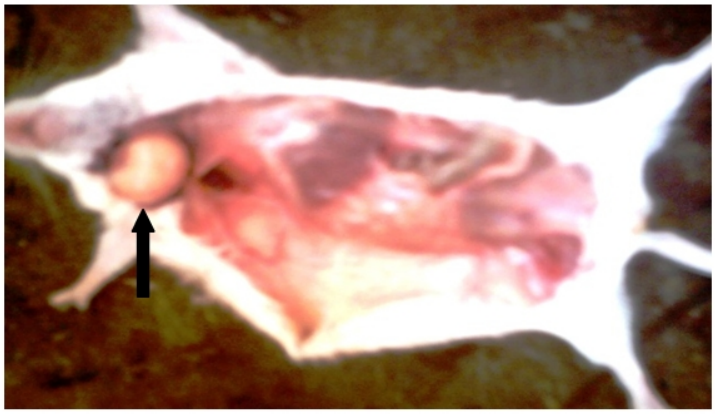

Fig. 12. Suspected tumor in the cervical region in Male mouse induced with MNU

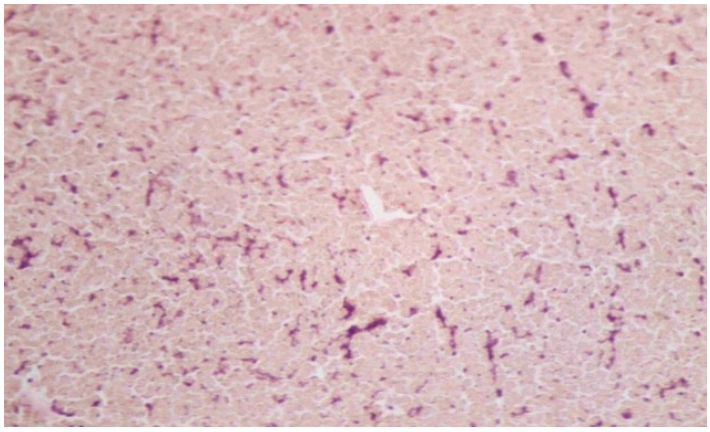

Fig. 13. Histological section of the above tumor showing necrosis (Mag. X 400)

3.3 Hematological Analysis

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

Table 1. Hematological Parameters of MNU induced and control Albino Mice

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_l	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±3.25	68.5±2.12	30.25±1.77	0.75±0.35	0.5±0.71	7.6±0.14
M_l	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±6.78	68±1.41	30±2.83	1.5±1.41	0.5±0.0	7.55±0.07

Fc-Female control; F_l- Female induced; Mc-Male control and M_l- Male induced; PCV- Packed Cell Volume; Hb- Hemoglobin; RBC - Red Blood Cell and WBC- White Blood Cell. (n=2)

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 [7]. 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 the authors [19]. 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 [20]. 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 [7]. 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 [11]. Increased lymphocytic activity was observed in the thymus of the female induced and the spleen of male induced mice respectively. Enlarged nuclei sizes were also observed in the thymus of the induced mice in the studies of Morton et al. [11]. 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 [21]. These authors also reported other degenerations in the heart which included myofibrosis, distortion of muscle fibres and mineralization. Splenic hemosiderosis, epitheliomorphic peritoneal mesothelioma and biphasic (solid epithelial and fusocellular pattern) were abnormalities observed in the spleen of an MNU induced rat [22]. However, there were no histopathological changes recorded in the heart and spleen of rats exposed to MNU intravenously in a six month study [23].

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 [24]. 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 [25]. Most tumor initiating agents either generate or are metabolically converted to electrophilic reactants which bind covalently to cellular DNA [26,27]. Free radicals and these modified DNA bases have been strongly implicated in carcinogenesis in general [28,29]. A

high correlation has been found to occur between the dose of administrated 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 [30]. MNU is a well known liver carcinogen which induces multiple cellular, molecular, and biochemical changes [31].

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 (Fig. 9). 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 in albino rabbits [32]. The results of the histopathology of the liver were very consistent with early reports in F344/DuCrj rats and Crj: BDF1 mice [33]; and in male Wistar rats [34]. In rabbits, necrotic areas in the liver cells associated with vacuolar changes and proliferation of connective tissue fibres were also reported [35]. Some of these observations have also been reported in the liver of rats exposed to formaldehyde [36,37]. 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. Vacuolation in the posterior cortex of the lens of neo-natal Sprague Dawley rats was observed as cataract development progressed [12]. 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. These include the albino rabbit [32]; Wistar rats [7,34] and in P53+/- mice [11].

In comparison with other studies, MNU was administered to female Sprague Dawley rats at a dosage of 50 mg/kg in animals of age 50, 80 and 110 days old [4]. About 90% developed mammary tumors. A single intravenous dose of 50 mg/kg administered in 120 day old Sprague Dawley rats showed a cancer incidence of 75-95% which were mainly mammary carcinomas [3]. da Silva Franchi et al. [7] 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). Similarly Morton et al. [11] 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 intra-peritoneal injection of 100 mg/kg [12]. 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 [38,39]. 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 monocytes 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 that the ranges given above are not firm boundaries as age, sex, strain, sampling techniques and methodology may account for some degree of variation [38].

The results of the hematological analysis were then compared with studies by other authors [40,41] who observed significant differences in some hematological parameters between control and tumor bearing mice. Hemoglobin for instance, in the studies by Raman et al. [41] 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 ± 1.36% in control compared to tumor bearing mice with 26.2 ± 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. [8] at 24% and 31% for tumor bearing and control mice respectively were also lower than obtained in our study. Studies by Chakraborty et al. [40] showed a similar pattern as that of Raman et al. [41]. Lymphocytes in normal unexposed mice were 67.13 ± 1.41 compared to tumor bearing mice at 18.54 ± 0.28%, while red blood cell count in control and tumor bearing mice in studies of Sathistha et al. [19] 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 (Figs. 12 and 13) 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.

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.

ACKNOWLEDGEMENTS

One of us, Aderoju Osowole thanks TWAS-COMSTECH for the research grant 09-137 RG/PHA/AF/AC_C-UNESCO FR: 3240231226 used to purchase the chemicals for the study; and TWAS-DFG for a fellowship to Germany to undergo training in anti-cancer studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Macejova D, Brtko J. Chemically induced carcinogenesis: a comparison of 1-Methyl-1-Nitosourea, 7, 12- DiethylBenzanthracene, Diethylnitroso-amine and Azoxymethan Models (Minireview). Endocrine Regulations. 2001;35:53-9.
2. Grubbs CJ, Becci PJ, Thompson HJ, Moon RC. Carcinogenicity of N-methyl-N-nitrosourea and N-ethyl-N-nitrosourea when applied to a localized area of the hamster trachea. J. Natl. Cancer Inst. 1981;66:961-65.
3. Moon RC, Kelloff GF, Detrisac CJ, Steele VE, Thomas CF, Sigman CC. Chemoprevention of MNU-induced mammary tumours in the mature rat by 4-HPR and tamoxifen. Anticancer Res. 1992;12:1147-53. PUBMED ID:1386970.
4. Rivera ES, Andrade N, Martin G, Melito G, Cricco G, Mohamad N, et al. Induction of mammary tumors in rat by intraperitoneal injection of MNU: histopathology and estral cycle influence. Cancer Lett. 1994;86:223-28. PUBMED ID:7982211
5. Takahashi H, Uemura Y, Nakao I, Tsubura A. Induction of mammary carcinomas by the direct application of crystalline N-methyl-N-nitrisourea onto rat mammary gland. Cancer Lett. 1995;92:105-11. PUBMED ID:7757955.
6. Thompson HJ, McGinley JN, Rothhammer K, Singh M. Rapid induction of mammary intraductal proliferations, ductal carcinoma in situ and carcinomas by the injection of sexually immature female rats with 1-methyl-1-nitrosourea. Carcinogenesis.1995; 16:2407-411. PUBMED ID:7586143.
7. da Silva Franchi CA, Bacchi MM, Padovani CR, de Camargo JL. Thymic lymphomas in Wistar rats exposed to N-methyl-N-nitrosourea (MNU). Cancer Sci. 2003;94(3):240-43. PUBMED ID:12824916.
8. Akanni EO, Oloke JK, Fakunle EE. Haematological characterization in N-nitroso-N-ethylurea induced tumour bearing rats on oral administration of pleurotus pulmonarius and pleurotus ostreatus metabolites. IJRRAS. 2010;3(3):343-48.
9. Mori S, Takeuchi Y, Toyama M, Makino S, Ohhara T, Tochino Y. et al. Amitrole: Strain differences in morphological response of the liver following subchronic administration to mice. Toxicol. Lett. 1985;29(2-3):145-52. PUBMED ID:4089883.
10. Festing MFW. In bred strains should replace out bred stocks in toxicology, safety testing and drug development. Toxicol. Pathol. 2010;38:681-90. DOI:10.1177/0192623310373776.
11. Morton D, Bailey KL, Stout CL, Weaver RJ, White KA, Lorenzen MJ. et al. N-Methyl-N-Nitrosourea (MNU): A positive control chemical for p53+/- mouse carcinogenicity studies. Toxicol. Pathol. 2008;36:926-31. DOI:10.1177/0192623308324959.
12. Yoshizawa K, Oishi Y, Nambu H, Yamamoto D, Yang J, Senzaki H. et al. Cataractogenesis in Neonatal Sprague Dawley rats by N-Methyl-N-nitrosourea. Toxicol. Pathol. 2000;28:555-64. PUBMED ID:10930042.

13. Pazos P, Lanari C, Elizalde P, Montecchia F, Charreau EH, Molinolo AA. Promoter effect of Medroxyprogesterone Acetate (MPA) in N-methyl-N-nitrosourea (MNU) induced mammary tumors in BALB/c mice. Carcinogenesis 1998;19:529-31. PUBMED ID:9525291.
14. Thompson HJ, Adlakha H. Dose-responsive induction of mammary gland carcinomas by the intra-peritoneal injection of 1-Methy-1-nitrosourea. Cancer Res. 1991;51:3411-415. PUBMED ID:2054781.
15. Lu CC, Meistrich ML. Cytotoxic effects of chemotherapeutic drugs on mouse testis cells. Cancer Res.1979;39:3575-582. PUBMED ID:476683
16. Barres V, Ouellet V, Lafontaine J, Tonin PN, Provencher DM, Mes-Messon AM. An essential role for Ran GTPase in epithelial ovarian cancer cell survival. Molecular Cancer. 2010;9:272. PUBMED ID:20942967.
17. Moore R. The white blood cell count in the indigenous people of East Africa. J. Trop. Med. Hyg, 1958;61:70-2.
18. Stevens ML. Fundamentals of Clinical Hematology. In: Banaee M, Mirvagefei AR, Rafei GR. Majazi AB. Effect of sublethal diazinon concentration on blood plasma biochemistry. Int. J. Environ. Res. 2008;2:189-98.
19. Sathistha MP, Shetti UN, Revankar VK, Pai KSR. Synthesis and antitumor studies on novel Co(II), Ni(II) and Cu(II) metal complexes of bis(3-acetylcoumarin) thiocarbohydrazone. Eur. J. Med. Chem. 2008;43:2338-346. DOI:10.1016/j.ejmech.2007.10.003.
20. Pelgrom S, Lock R, Balm P, Wendelaar Bonga S. Integrated physiological response of tilapia, *Oreochromis mosssambicus* to sublethal copper exposure. Aquat. Toxicol. 1995;32:303-20.
21. Palazzi X, Kergozien-Framery S. Use of rasH2 transgenic mice for carcinogenesis testing of medical implants. Exp. Toxicol. Pathol. 2009;61:433-41.
22. Minardi F, Maltoni C. Results of long-term carcinogenicity bioassays of ceramic fibres (“Fiberfrax”) on Sprague-Dawley rats. Eur. J. Oncol. 1998;3:241-49.
23. Murata S, Kominsky SL, Vali M, Zhang Z, Garrett-Mayer E, Korz D. et al. Ductal Access for Prevention and Therapy of Mammary Tumors. Cancer Res. 2006;66(2):638-45. DOI:10.1158/0008-5472.CAN-05-4329.
24. Fazeli SA, Davarian A, Azarhoush R., Golalipour MJ. Histopathologic changes of rat liver following formaldehyde exposure. Pak. J. Biol. Sci. 2006;9:2137-140.
25. Ward JM, Shibata MA, Devor DE. Emerging issues in mouse liver carcinogenesis. Toxicol. Pathol. 1996;24:129-37. PUBMED ID:8839290.
26. Slaga TJ, O' Connell J, Rostein J, Patskan G, Morris R, Aldaz CM. et al. Critical genetic determinants and molecular events in multistage skin carcinogenesis. Symp. Fundam. Cancer Res. 1986;39:31-44. PUBMED ID:3321308.
27. DiGiovanni J. Multistage carcinogenesis in mouse skin. Pharmacol Ther. 1992;54:63-128. PUBMED ID:1528955.
28. Floyd RA. Role of oxygen free radicals in carcinogenesis and brain ischemia. Fed Am. Soc. Exp. Biol. J. 1990;4:2587-597.
29. Malins DC. Identification of hydroxyl radical lesions in DNA base structure: biomarkers with a putative link to cancer developments. J. Toxicol Environ Health. 1993;40:247-61. PUBMED ID:8230300.
30. Izzotti A, Camoirano A, Cartiglia C, Grubbs CJ, Lubet RA, Kelloff GJ. et al. Patterns of DNA adduct formation in liver and mammary epithelial cells in rats treated with 7,12-Dimethyl-benz(a)anthracene, and selective effects of chemopreventive agents. Cancer Res. 1999;59:4285-290. PUBMED ID:10485473.
31. Samaras V, Rafailidis PI, Mourtzoukou EG, Peppas G, Falagas ME. Chronic bacterial and parasitic infections and cancer: a review. J. Infect. Dev. Ctries. 2010;4:267–81.

32. Shashi A, Thapar SP. Histopathology of fluoride-induced hepatotoxicity in rabbits. Fluoride. 2000;34:34-42.
33. Yamamoto S, Kasai T, Matsumoto M, Nishizawa T, Arito H, Nagano K. et al. Carcinogenicity and chronic toxicity in rats and mice exposed to chloroform by inhalation. J. Occup. Health. 2002;44:288-93.
34. Brzoska MM, Moniuszko-Jakoniuk J, Pilat-Marcinkiewicz B, Sawicki B. Liver and kidney function and histology in rats exposed to cadmium and ethanol. Alcohol Alcohol, 2003;38(1):2-10. DOI:10.1093/alcalc/agg006.
35. Wang J, Zheng ZA, Zhang LS, Cao DM, Chen KZ, Lu D. An experimental study for early diagnostic features in fluorosis. Fluoride. 1993;26:61-5.
36. Bandman A. Hazardous substances, galogen and oxygen containing substances. Chimia. 1994;48:124-28.
37. Teng S, Beard K, Pourahmad J, Morideni M, Easson E, Poon R. et al. The formaldehyde metabolic detoxification enzyme systems and molecular cytotoxic mechanism in isolated rat hepatocytes. Chem-Biol. Interact. 2001;130-132:285-96. PUBMED ID:11306052.
38. University of Minnesota, Research Animal Resources. Reference values for laboratory animals. Normal Hematology Values. Accessed 17 February, 2012.
Available: http://www.ahc.umn.edu/rar/refvalues.html
39. Iowa State University. Vice President for Research and Development. Normal values in the mouse. Hematology. Accessed 17 February, 2012.
Available: http://www.lar.iastate.edu/index.php?option=com_content&
40. Chakraborty A, Kumar P, Ghosh K, Roy P. Evaluation of a Schiff base copper complex compound as potent anticancer molecule with multiple targets of action. Eur. J. Pharmacol. 2010;647:1-12.
41. Raman N, Jeyamurugan R, Senthilkumar R, Rajkapoor B, Franzblau SG. In vivo and in vitro evaluation of highly specific thiolate carrier group (II) and Zinc (II) complexes on Ehrlich ascites carcinoma tumor model. Eur. J. Med. Chem. 2010;45:5438-451. DOI:10.1016/j.ejmech.2010.09.004.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=160&id=5&aid=739