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# THE CARCINOGEN EFFECTS OF DIMETHYLAMINOAZOBENZEN (DMAB) DYE ON THE SKIN OF RATS

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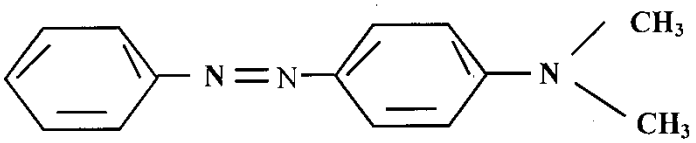
**Key words:** DMAB,Carcinogenic effect,Squamous cell carcinoma.

## ABSTRACT

The effects of dimethylaminoazobenzene dye were studied in rats by exposing skin directly to the dye. Twenty one mature rats Rattus norvegicus were divided into three groups and represented by (control group ; T1group treated with ether (vehicle) only, and T2 group treated with dimethylaminoazobenzene dissolved in ether). The obtained results concluded the carcinogenic effects of dimethylaminoazobenzene depending on marked grossly and histological changes represented by appearance of skin cancer (squamous cell carcinoma and basal cell carcinoma) types in T2 group.

## INTRODUCTION

Although exposure to chemical agents undoubtedly plays a significant role in the causation of some neoplastic diseases, there is no scientific basis on which to calculate that particular percentage of human cancers is due solely or principally to chemicals(1). The suggestion frequently made and pointed that more than 80% of human cancers are contribute to environmental rather than to factors is not founded on substantial fact ; and even if they were true, the term environmental factors includes physical and viral as well as chemicals (2). One of the most common chemicals substance that release to the environment is 4-imethylaminoazobenzene ; this soapstone have many synonyms : as N , N dimethyl – p - phenylazoaniline, p - dimethylaminoazobenzene, C. I. solvent yellow 2, methyl yellow, outlast yellow R, brilliant fast oil yellow, brilliant fast spirit yellow, butter yellow, cerasine yellow GG, C. I. 11020, DAB, dimethyl yellow, DMAB, enial yellow 2G, fat yellow, oil yellow, sudan yellow, sudan gg, waxoline yellow ad, numerous further trade names (3,4,5,6,7). The dye molecular formula is : (8)



60-11-7 C.A. Registry

Molecular formula C14H15N3, 11020 C.I

Molecular weight, M.W 225.29, percent composition 74.64%C,

18.65% N, 6.71% H

Release of dimethylaminoazobenzene to the environment may occur as a result of its manufacture and use as a dye intermediate, in photosensitive polymers and reusable films, as an indicator in volumetric analysis, in tests for oxidized fat, and as a coloring agent (9,10). If it is released to soil it may bind to the soil based on an estimated Koc of 7390 and therefore should not leach to the groundwater (11,12.13). However, since it has a pKa of 3.226 at 25 C, it exists partially as a action and the extent of its adsorption to soils and sediments should be affected by the pH of the medium (14, 15, 16). (17) shows in study of dimethylaminoazobenzene properties it is not hydrolyze in soils, no information was found on its biodegradation in soils, if it is released to water it may bioconcentrate in aquatic organisms, adsorb to sediment, and may be subject to direct photolysis and hydrolyze and evaporate from water. Based on a laboratory-screening test using an inoculum's from settled domestic wastewater, it may be subject to biodegradation. It is released to the atmosphere, and it may be a subject to direct photolysis and the estimated vapor phase half-life in the atmosphere is 7.04 hr as a result of photo chemically produced hydroxyl radicals adding to the aromatic rings ; however, it may exist primarily adsorbed on to particulate matter due to its very low vapor pressure (18,19,20). (21) refered that dimethylaminoazobenzene dye is used as a dye for coloring polishes, wax products, and soap. Acute (short - term) dermal exposure to 4-dimethylaminoazobenzenemay result in contact dermatitis in humans. While (21) fixed no effects is available on the chronic (long - term), reproductive, developmental, or carcinogenic effects of dimethylaminoazobenzene in humans but in animal studies have reported birth defects in the offspring of mice exposed to 4-dimethylaminoazobenzene and tumors of the lung, liver, and bladder from oral exposure to 4-dimethylaminoazobenzene (22,23). (24) has not classified 4-dimethylaminoazobenzene for carcinogenicity ; While (25)has classified 4-dimethylaminoazobenzene as a Group 2B, possibly carcinogenic to humans. Exposure may occur as a result of occupational dermal exposure. Rats have fewer and smaller GST-P (glutathione – S – transferase - placental form) than F344 so are highly resistant to chemical

induction of hepatocellular carcinoma and preneoplastic lesions, but when the male rats were treated with N - nitrosodiethylamine and fed a diet containing 3 – Me - DAB, after 8 weeks had less than 4% glutathione S - transferase placental form (GST - P) positive lesions, this suggested the suppression of positive foci in the liver under these conditions relative liver weight of animals injected with N - nitrosodiethylamine (DEN) and given 3 – Me - DAB diet was less than rats treated the same way (26)

## MATERIALS AND METHODS

A total of 21 mature rats Rattus.norvegicus purchased from drug control center (Baghdad)were housed in metal cage and given tap water and food pellets adlibitum. The temperature was maintained at 25 ± 2 relative humidity 40 – 70 % and light/dark cycle of 12h. Rats were divided in to three groups of equal number each group represented by:

1-Control group not exposed to any things

2-T 1group was exposed to ether by wipe some areas of skin (back, abdomen and tail) with ether weekly for 28weeks.

3-T2group was exposed to dimethylaminoazobenzene that dissolved in ether in concentration 250mg / 1 ether, by wipe some areas of skin (back, abdomen and tail) with dimethylaminoazobenzene dissolved in ether weekly for28weeks.

Samples of the skin were tack from experiment animals after remarkable external changes were apparent represented by remarkable scars and warts on back, abdomen and tail, which gave a simple guide to the progress of experiment.

The experiment continued 28 week and the rats were killed and dissected at experiment terminated and the skin specimens rinsed thoroughly in normal saline. The skins were fixed, embedded and stained with hematoxyline eosin stain according to (27).

## RESULTS AND DISCUSSION

Epidermis is the outermost layer of the skin. It forms the waterproof, protective wrap over the body's surface and is made up of stratified squamous epithelium with an underlying basement membrane. It contains no blood vessels, and is nourished by diffusion from the dermis. The main type of cells which make up the epidermis are keratinocytes, with melanocytes also present. Epidermis is divided into several layers where cells are formed through mitosis at the innermost layers. They move up the strata changing shape and composition as they differentiate and become filled with keratin. They eventually reach the top layer called stratum corneum and become sloughed off, or desquamated. This process is called keratinization and takes place within weeks. Epidermis is divided into the following 5 sublayers or strata : Stratum corneum

Stratum lucidum Stratum granulosum Stratum spinosum Stratum germinativum also called stratum basale. The inner layer, the dermis conssist of irregular conneactive tissue in which many hair follicals are scattered. (28)

In this study the resultes showed non grossly and microscopicaly changes in all skin specimens that collected from control group and T1group which exposed to ether only along the experment period (Fig-1,2). While in T2 group which exposed to dimethylaminoazobenzene that dissolved in ether along the experment, the grossly examinationwas showed signs of inflammation,rashes, an open sore, reddish patch, swollen and scalp in the all area that exposed to dimethylaminoazobenzenefrom starting experment untile the progressed case apperant as scare, shiny bump and warte (Fig, 3, 4, 5).

Furthermore microscopical examination results was showed changes are represented by compact areas, well delineated and invading the dermis, apparent with no connection with the epidermis. tumor cells resemble normal basal cells (small, monomorphous or their disposal tends to be similar to that of normal epidermis : immature/basal cells at the periphery, becoming more mature to the centre of the tumor masses), are disposed in palisade at the periphery of the tumor nests ; but are spindle - shaped and irregular in the middle ; tumor clusters are separated by a reduced stroma with inflammatory infiltrate (The surrounding stroma is reduced and contains inflammatory infiltrate (lymphocytes) ; all this histological changes are fix as Basal cells carcinomas (BCC). (Fig - 6, 7, 8, 9).

The grossly and microscopical examination results in this study was diagnosed another type of skin cancer represented by Squamous cell carcinoma (SCC), characterized by scaly red patch with irregular borders that sometimes crusts or bleeds. Differentiated diagnosis of squamous cell carcinomas compared with basal cells carcinomas, the first contain more pleomorphic cells and no keratinization tumor cells transform into keratinized squames and form round nodules with concentric, laminated layers, called cell nestsor epithelial / keratinous pearls (Fig - 10, 11).

Basal cell carcinoma and Squamous cell carcinoma are the most common form of skin cancer, affecting 70% of patina of cancer each year. In fact, it is the most common of all cancers. One out of every three new cancers is a skin cancer, and the vast majorities are basal cell carcinoma than squamous cell carcinoma, often referred to by the abbreviation, BCC SCC. These cancers arise in the basal cells, which are at the bottom of the epidermis (outer skin layer). Until recently, those most often affected were older people, particularly men who had worked outdoors. Although the number of new cases has increased sharply each year in the last few decades, the average age of onset of the disease has steadily decreased. More women are getting BCC than in the past ; nonetheless, men still outnumber them greatly (29).

The major causes are chronic exposure to sunlight and most chemical particles such as arsenic, benzene (hydrocarbons compound), coal, exposure that which occur most frequently on exposed parts of the body the face, ears, neck, scalp, shoulders, and back. Rarely, however, tumors develop on non - exposed areas. In a few cases, contact with to radiation, and complications of burns, scars, vaccinations, or even tattoos are contributing factors (30, 31, 32). Furthermore the sequel of chronic inflammation such as chronic ulcers, granulomas and chronic irritation led to appearance and development of skin cancer (33, 34).

Carcinogenic polycyclic hydrocarbons are considered initiaters, producing an irreversible change in the cells of the target tissue (35).

The 4 - dimethylaminoazobenzene dye virulence appears under penetrable the skin and accumulated in hair follicles and sebaceous glands induced necrotic lesions led to death of cells and a stimulate hyperplasia which then developed to cancer (36).

Nature of the carcinogenic hazards of the carcinogen including local and systemic toxicity, so one of serious characteristic of this dye produced free radicals such as alkyl group, methyl group, nitrose and benzene rings which act as a mutagens that play important role by chemically modifying base resulting in an alteration of base pair from Guanine - cytosine in to Adenine - Thiamine, this mutation led to errors introduced during DNA replication and the sequel produced cancerous cells developed into cancer and this results agreement with the finding of (37, 38) who declared that DNA is primary target for chemical carcinogen and its metabolite.

**Fig. 1;** Control group(280x) H&E

**Fig2:**T1 Group(250x) H&E

Fig (1 and 2) normal microscopical examination of skin specimens that collected from control group and T1group, which exposed to ether only along the experment period

**Fig.3 ;** T2Group

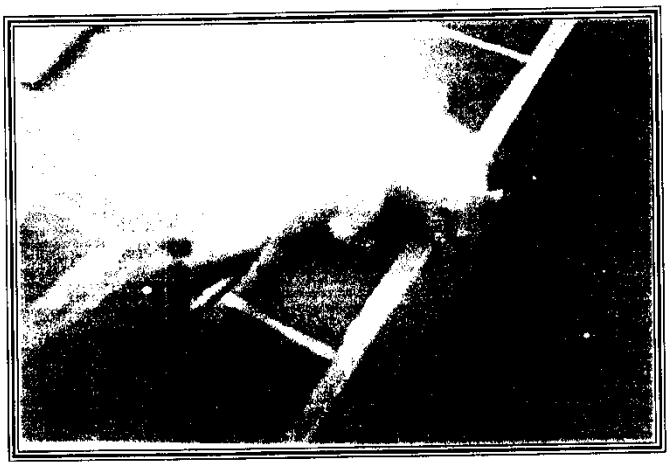


**Fig. 4;** T2 Group

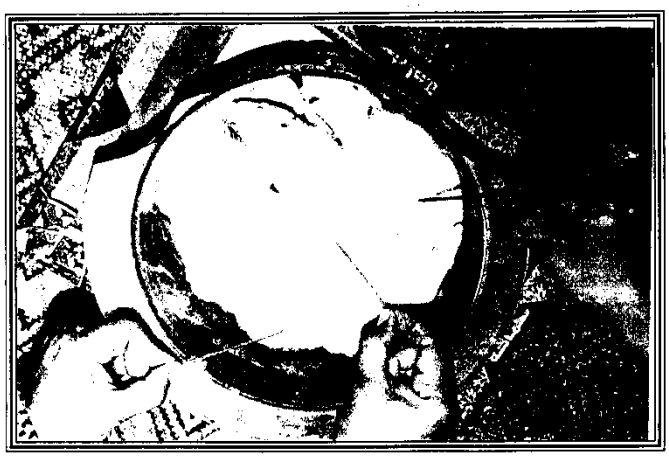


**Fig. 5;** T2 Group

Fig (3, 4, and 5) grossly examination of T2 group skin, showed signs of inflammation, rashes, an open sore, reddish patch, swollen and scalp in the all area that exposed to (DMAB) from starting experment untile the progressed case apperant as scare,shiny bump and warte.

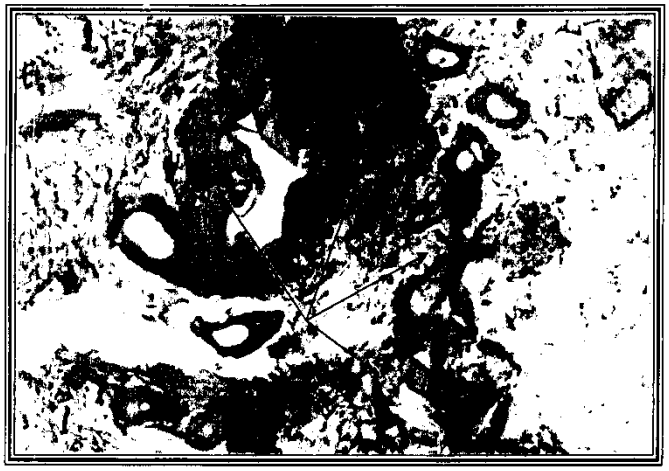


**Fig.6;** T2 Group(250x) H&E

**Fig7:** T2 Group(250x) H&E

**Fig8;** T2 Group(250x) H&E

**Fig.9**: T2 Group(250x)H&E

Fig (6, 7, 8, and 9) microscopical examination of T2 group skin specimens showed many changes represented by compact areas, well delineated and invading the dermis, apparent with no connection with the epidermis. tumor cells resemble normal basal cells, and disposed in palisade at the periphery of the tumor nests ; but are spindle-shaped and irregular in the middle; tumor clusters are separated by a reduced stroma with inflammatory infiltrate (The surrounding stroma is reduced and contains inflammatory infiltrate (lymphocytes);all this histological changes are fix as Basal cells carcinomas (BCC)

**Fig.10;** T2 Group(250x) H&E

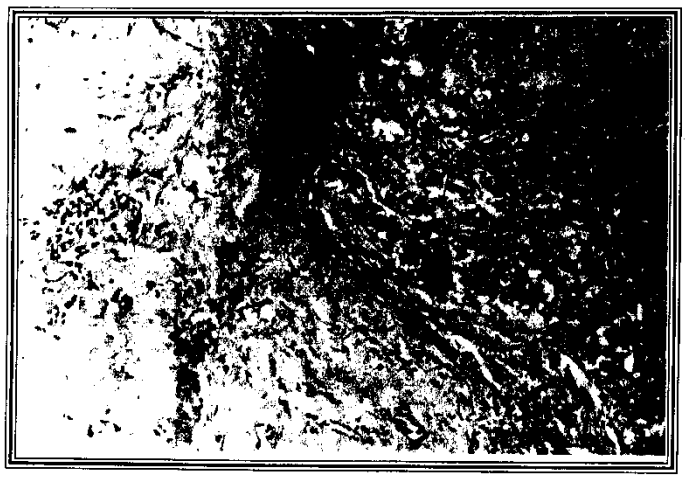


**Fig. 11:** T2 Group (250x)H&E

**Fig 12;**T2 Group(250x)H&E

Fig (10, 11, and 12) microscopical examination of T2 group skin specimens showed another type of skin cancer represented by Squamous cell carcinoma (SCC), characterized by scaly red patch with irregular borders that sometimes crusts or bleeds ; contain more pleomorphic cells and no keratinization tumor cells transform into keratinized squames and form round nodules with concentric, laminated layers, called cell nestsor epithelial / keratinous pearls

## التأثيرات المسرطنة لصبغةDIMETHYLAMINOAZOBENZEN(DMAB) على الجلد في الجرذان

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**ألخلاصه**

درست تأثيرات الصبغة dimethylaminoazobenzene في الجرذان بتعريض الجلد مباشرة للصبغة .21 جرذ بالغ من سلالةRattus norvegicus قد قسمت إلى ثلاث مجاميع تمثلت (بمجموعة السيطرة ومجموعةT1عوملت بالايثر كحامل دوائي فقط و مجموعة T2 عوملت بالصبغةdimethylaminoazobenzene المذابة في الايثر ) استنتاجات النتائج بالتأثيرات المسرطنة للصبغة اعتمدت على التغيرات العيانية والنسيجية الواضحة المتمثلة بظهور سرطان الجلد نوع سرطانةالخلايا الحرشفية وسرطانة الخلايا القاعدية في مجموعة T2.

## REFERENCES

1- Magee, P. N. (1981). Some illustrative system of chemical carcinogenesis Nitrosamine and hydrocarbons. Sci. Found. of oncology. 5 (3) : 192 - 200.

2- C. N. R. E. A. Cancer Research (1991). Public. Ledger Building Philadelphia. 51 : 2040 (abst).

3- N. I. O. S. H. (National Institute for Occupational Safety and Health) (2000). The registry of toxic effects of chemical substance. 23 : 1 – 12.

4- N. O. E. S. (National Occupational Exposure survey) (1993). Elsevier science pub. Co. Inc. Ney york.

5- Kamrin, M. (1980). Hazardous substance. Nucl. Chem. J. 80 (5) : 1368 – 1372.

6- E. N. M. U. D. M. (Environmental Mutagenesis) (1996). New York pub. In formation (Alan R. Liss). 16 : 1453.

7- D. C. T. O. D. Drug and chemical Toxicology (1985). Madison Ave. New York. 78 : 220 (abst.).

8- Miller, G. E.; Croffet, T. A., Manea, M. C. and Nault, M. (1992). Chemical composition and properties of DMAB in existence. Radional. Nucl. Chem. 160 (3) : 3682 – 3689.

9- N. O. H. S. (National Occupational Hazard survey) (1989). Washington D. C. 9 : 862 – 875.

10- Clayson, D. B.(1978). Chemicals and Environmental carcinogenesis in man. Enr. J. Cancer 3 : 405.

11- Clark, C. H. (1992). Metabolic effect and toxicities of (DMAB) in dogs eating high protein diet. Br. Vet. Med. J. 142 (5) : 836 – 841.

12- Bogdan, I. (1994). Chemical stability storage quantities determination and identification of some hazardous pollutants. J. Biol. Chem.. 188 (7) : 1612 – 1618.

13- Bartsch, H. and Grover, P. L. (1996). Chemical and physical carcinogenesis and mutation. cancer Res. J. 31 (6) : 1016 – 1022

14- Bandura, S. R. ; Armstrong. B. and Doll, R. (1993). Environmental Factors and cancer incidence and mortality in different countries with special reference to dietary practicles. Int. J. Cancer. 33 (3) : 817 – 831.

15- Anghileri, E. S. ; Miller. J. R. ; Robinette, K. N. ; Prasad, K. and Lagrebory, M. (1991). The biological effect of environmental pollutants. Nat. Acad. Of Sci. Midline (abst).

16- Glazko, A. J. (1986). Effect of (DMAB) on liver function Amer. J. Med. 96 : 722 – 726.

17- Ames, B. N. (1989). Mutagenesis and carcinogenesis : Endogenous factors. Environ. Molec. Mut. 14 (16) : 66 – 77.

18- T. J. A. D. A. B. (Teratology, The International Journal of Abnormal Development) (1990). Alan. R. Liss, Inc. New York. 12.

19- T. C. M. U. D. (Teratogenesis, carcinogenesis and mutagenesis) (1981). Alan R. Liss, Inc, New York. 12.

20- Hughesy, B. W. (2000). Studies on (DMAB) possible determinates and progress of haemopoietic toxicity during (DMAB) exposure. Med. J. Austin. 62 (6) : 1142 – 1149.

21- Janardhan, A. and Kumer, S. (1988). Teratogenicity of methyl benzimi – dazole carbamata in rats and rabbits. Bull. Environ. Contam. Toxicol, 33 : 257 – 263.

22- E. P. A. (Environmental Protection Agency) (1986). 16 : 1112 – 1143.

23- Bishop, J. M. (1989). The molecular genetics changes by DMAB in experimental animal. J. Natl. Cancer. Inst. 27 (5) : 971 – 982.

24- Elizabeth, K. W. (1989). Techniques for carcinogenicity studies. Laboratory of carcinogen metabolism, Nat. Cancer. Ins. Bethesda. Maryland. 72 (8) : 753 – 762..

25- M. B. E. E. P. S. (Medical and Biological Effect of Environmental pollutants Series) (2003). Washington, D. C. Natl. Acd. Of Sci. 79.

26- Farcoun, F. I. (1996). Bioactivities of heterocyclic amine by formation toxic glutathione conjugated. Chem. Index. 11 : 107 – 136.

27- Luna, L. G. (1968). Manual of histology stain method of armel force institute pathology, 3rded. The Blackstone diction McGraw Hill book. Comp. New York, London and Sydney.

28- Anderson, J. R. (1986). Muir's textbook of pathology. 12thed . University of Glasgow. By Edward Arnold (publishers) ltd. 41 Bed ford square. London.

29- Marks,R.(1996). Suquamous cell carcinoma. Lancet. 347 : 735 - 738.

30- Coggon. D. (1999) Occupational cancer in the United Kingdom. Environ Health Perspect. 107 (2) 239 - 244.

31- Solan, M. J. ; Brady, L. W. (2001). Skin cancer. In : Clinical Oncology 8thed. Rubin, L. Company. Philadephia, USA. 252 - 266.

32- Marks, R. (1995). An over view of skin cancers. Incidences and causation. cancer. 75 : 607 - 612.

33- Moulton, J. E. (1987). Tumors in domestic animals. 2nded University of California press. 1td London, England.

34- Karagas, M. R . ; Greenberg, E. R. ; Spenger, S. K. (1999). Increase in incidence rates of basalcell and sequamus cell skin cancer in New Hampshier, USA. Int. J. Cancer 85 : 557 - 561.

35- Miller, E. C. and Miller, J. A. (1979). The metabolism of carcinogens to reactive electrophiles and their possible mechanism of action in carcinogenesis in E. S. Seart. che. carci. A. C. S Monograph No. 173 : 737

36- Potten, C. S. (1974). The epidermal proliferation In : J. F. Lamerton (1993). Cell population kinetic in normal and malignant tissue cancer Res. 13 : 114 – 125.

37- Frindel, E. ; Charnyer, F. ; Kaplan, H. S. and Alpen, E. L. (1989). Radiation effect on DNA synthesis and cell division in bone marrow of mice. Cancer . Res. 25 (1) 314 – 319.

38- Shank, R. C. , and Barrows, L. R. (1981). Tumor dependant DNA methylation significant to risk assessment Frankli, Institute : 225.