

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

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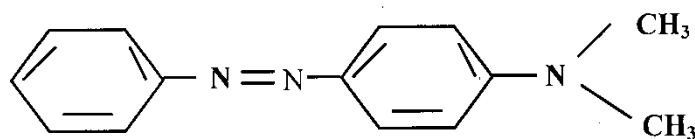
Key words: DMAB, Carcinogenic effect, Squamous cell carcinoma.

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 ; T1 group 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-dimethylaminoazobenzene ; 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 K_{oc} of 7390 and therefore should not leach to the groundwater (11,12,13). However, since it has a pK_a of 3.226 at 25 °C, it exists partially as a cation 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 photochemically 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) referred that dimethylaminoazobenzene dye is used as a dye for coloring polishes, wax products, and soap.

Acute (short - term) dermal exposure to

4-dimethylaminoazobenzene may 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 / l ether, by wipe some areas of skin (back, abdomen and tail) with dimethylaminoazobenzene dissolved in ether weekly for 28weeks.

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 consist of irregular connective tissue in which many hair follicles are scattered. (28)

In this study the results showed non grossly and microscopically changes in all skin specimens that collected from control group and T1 group which exposed to ether only along the experiment period (Fig-1,2). While in T2 group which exposed to dimethylaminoazobenzene that dissolved in ether along the experiment, the grossly examination was showed signs of inflammation, rashes, an open sore, reddish patch, swollen and scalp in the all area that exposed to dimethylaminoazobenzene from starting experiment until the progressed case appeared as scab, shiny bump and wart (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 nests or epithelial / keratinous pearls (Fig - 10, 11).

Basal cell carcinoma and Squamous cell carcinoma are the most common form of skin cancer, affecting 70% of patients 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 majority 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 initiators, 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, nitro 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

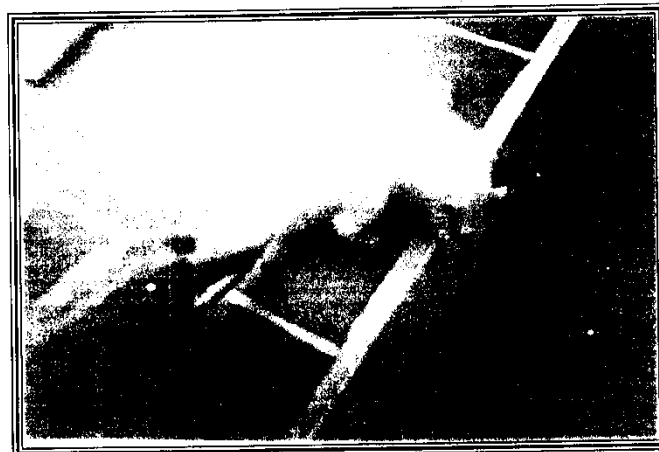


Fig. 4; T2 Group

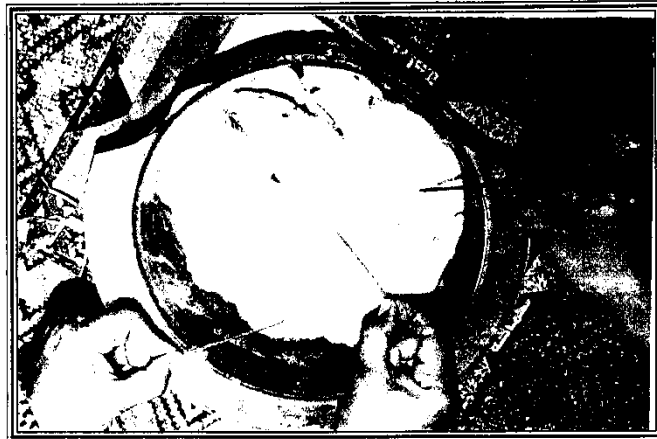


Fig. 5; T2 Group

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

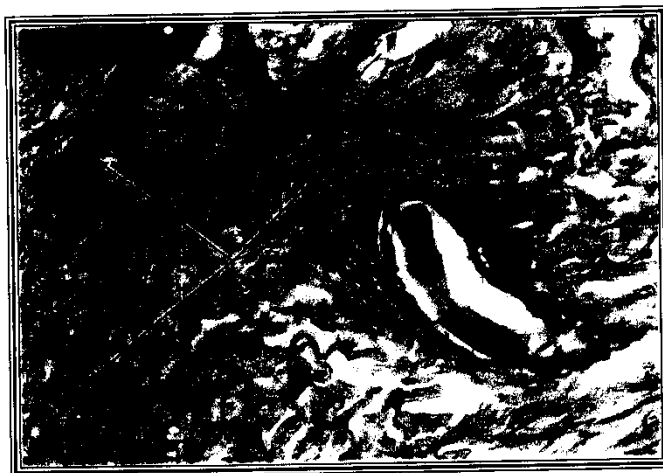


Fig8; T2 Group(250x) H&E



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

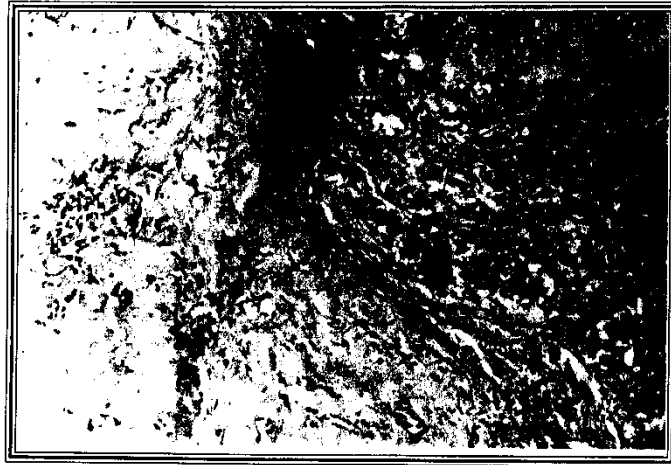


Fig. 11: T2 Group (250x)H&E



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 nests or epithelial / keratinous pearls

التأثيرات المسرطنة لصبغة (DMAB) DIMETHYLAMINOAZOBENZENE على الجلد في الجرذان

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الخلاصة

درست تأثيرات الصبغة dimethylaminoazobenzene في الجرذان بتعريض الجلد مباشرة للصبغة . 21 جرذ بالغ من سلالة *Rattus norvegicus* قد قسمت إلى ثلاث مجاميع تمثلت (بمجموعة السيطرة ومجموعة T1 عوملت بالايثر كحامل دوائي فقط و مجموعة T2 عوملت بالصبغة dimethylaminoazobenzene . المذابة في الايثر) استنتاجات النتائج بالتأثيرات المسرطنة للصبغة اعتمدت على التغيرات العيانية والنسجية الواضحة المتمثلة بظهور سرطان الجلد نوع سرطانة الخلايا الحرشفية وسرطانة الخلايا القاعدية في مجموعة T2.

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