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Exploring the effect of DMBA on haematological and biochemical parameters in rats

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Abstract
7, 12-Dimethylbenz (a) anthracene (DMBA), a polycyclic aromatic hydrocarbon, is a potent organ-specific carcinogen widely employed in experimental cancer research to induce mammary tumors in laboratory animals. This study aimed to investigate the hematological and biochemical alterations following oral administration of DMBA in female Sprague-Dawley rats. Rats were administered 20 mg/kg DMBA orally for four consecutive weeks, and tumor development was monitored over 60 days. Hematological and biochemical parameters were evaluated to assess physiological changes. DMBA exposure resulted in significant deviations in RBC, WBC, platelet counts, and liver and kidney function markers, suggesting systemic toxicity and tumor development. The study provides a foundation for evaluating therapeutic interventions against DMBA-induced carcinogenesis. DMBA (7, 12-Dimethylbenz (a) anthracene) is a potent, organ-specific laboratory carcinogen widely used in cancer research. It acts as a tumor initiator by inducing the necessary cellular alterations. The induction of mammary carcinomas in rats by chemical carcinogens, particularly DMBA, serves as an effective model for studying carcinogen sensitivity. DMBA belongs to a class of chemical carcinogens known as polycyclic aromatic hydrocarbons (PAHs). Most PAHs are persistent in the environment and bioaccumulate, disrupting biological balance and contaminating ecosystems. PAHs can cause cancer in various animal organs regardless of the delivery route. Furthermore, DMBA exposure leads to significant behavioral abnormalities and physiological disease processes, including cancer and aging.

Keywords: DMBA, SD rats, tumour, haematology, biochemistry

Introduction
The oxidative by-products of DMBA metabolism can damage proteins and lipid membranes, impairing physiological functions and leading to leukaemia and anaemia (Al-Asady *et al*., 2020) [1]. Reactive oxygen species (ROS) play a role in several disorders, and DMBA exposure in rats has been linked to increased levels of monocytes, eosinophils, neutrophils, and total leukocyte count (WBC), alongside elevated lipid peroxidation and oxygen radicals (Al-Asady *et al*., 2020) [1]. Contrasting these findings, Prakash *et al*. (2023) [14] observed that DMBA-treated rats had significantly lower red blood cell counts, white blood cell counts, platelet counts, and hemoglobin percentages compared to controls. However, *Mangifera indica* leaf extract significantly normalized these variables (p<0.05), (Prakash *et al*., 2023) [14]. Urkude *et al*. (2023) [23] also reported significantly lower TEC, Hb, and PCV in MNU-administered animals, indicating erythrocytopenia/anemia, thrombocytopenia, and leucocytopenia. Hesperidin treatment (160 mg/kg) restored these levels, while a lower dose (80 mg/kg) showed improved values compared to the untreated group (Urkude *et al*., 2023) [23].
DMBA administration leads to increased levels of biochemical parameters such as SGOT, SGPT, ALP, BUN, and creatinine (Ozdemir *et al*., 2007; Salem *et al*., 2004) [13, 16]. Salem *et al*. (2004) [16] further noted that these elevations were linked to pathological changes in the liver, mammary gland, kidney, heart, skeletal muscle, and erythrocytes, suggesting DMBA-induced organ damage. Suzuki *et al*. (2003) [17, 18] confirmed altered haematological and biochemical parameters with DMBA treatment, establishing its role in causing hepatocellular carcinoma, as well as skin, oral, mammary, and ovarian tumors.

Tominaga *et al*. (1970) [20] and Urano *et al*. (1973) [21] found that after bleomycin administration, its highest concentrations were in the mammary gland, followed by the lung, stomach, skin, and kidney. Bleomycin treatment initially increased WBC count, which then returned to near normal, while RBC count remained constant. A gradual decline in tumor size and significant reductions in DMBA-induced serum SGOT, SGPT, ALP, BUN, and Creatinine levels were observed following bleomycin treatment, indicating its effectiveness against DMBA-induced mammary tumors (Tominaga *et al*., 1970; Urano *et al*., 1973) [20, 21]. Similarly, oral administration of catechin (20 mg/kg) in rats reduced DMBA-induced elevated WBCs and normalized biochemical parameters like SGOT, SGPT, ALP, BUN, and Creatinine (Crespy & Williamson, 2004) [3].

In view of this study was planned to investigate effect of oral administration of DMBA in SD rats.

Material and Methods

Material

Experimental Animals

Virgin female Sprague-Dawley rats (N=48) weighing 150±10 g and aged 45-55 days were obtained from National Institute of Biosciences, Bhor Dist-Pune, India. All the experimental protocols used in this study were approved by the Institutional Animal Ethics Committee IAEC/26/24/KNPCVS/2024 and CCSEA, New Delhi (309/GO/ReRcBi-L/2000/CCSEA Dated: 15/12/2000. The animals were caged group wise and the identification of animals was done with the help of picric acid in Central Laboratory Animal House, Department of Veterinary Pharmacology and Toxicology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara-412801(Maharashtra). The laboratory animal facility is approved by CCSEA and the Institutional Animal Ethics Committee (IAEC) and the CCSEA Registration Number is 309/GO/ReRcBi-L/2000/CCSEA.

Housing

Animals were provided with a 12-hour light and 12-hour dark period and maintained under constant environmental conditions with an ambient room temperature of 23±2 °C and relative humidity between 55-65%. All the animals were maintained in hygienic conditions in polypropylene cages, (47X34X18 cm) lined with clean paddy husk as a bedding material. In each cage four animals were kept. Female SD rats were kept under constant observation for seven days of acclimatization before the commencement of the experiment. All necessary managemental procedures were adopted to keep the animals free from stress.

Feeding

The animals were fed with a standard pelleted diet, procured from the National Institute of Bioscience, Bhor and Pune. The pelleted diet consists of 22.02% crude protein, 4.25% crude oil, 3.02% crude fibre, 7.5% ash and 1.38% sand silica. Animals were provided with *ad lib* feed and wholesome pure drinking water throughout the period of experiment. Water bottles were cleaned with detergent and rinsed with hot water at least twice a week to maintain cleanliness.

Bedding material

Clean, dried and sterilised paddy husk was used as a bedding material for the experimental animals. Bedding material was changed every alternate day in order to maintain hygienic conditions.

Clinical signs and body weight

All the animals were monitored thrice daily (morning, afternoon and evening) for clinical signs such as diarrhoea, limping due to tumor development, abnormal behaviour and ill health. The body weight of all the rats was taken twice a month to monitor their feed intake.

Drugs and Chemicals

DMBA (7-12-Dimethylbenzanthracene) with CAS No.: 57-97-6 and Purity (GC): 98.0% was procured from Tokyo Chemical Industry (India) Pvt. Ltd. India. Tamoxifen Citrate as anticancer drug by Healing Pharma India Pvt. Ltd was procured from Saaj Medical Store, Shirwal. Methanol was procured from Hi-AR™ (HiMedia Laboratories Pvt. Ltd. Thane, India).CA 15-3 Elisa Calbiotech Kit was procured from Calbiotech, New Delhi. Biochemical kits were procured from PV Enterprises, Pune.

Method of DMBA Administration

For tumour induction, 20 mg/kg of DMBA, dissolved in olive oil, was administered intragastrically (orally) via gavage once a week for four consecutive weeks to 45- to 55-day-old female SD rats weighing 140±10 g. DMBA typically induces tumors in this model within 8-10 weeks. After DMBA administration, rats were monitored daily for tumor development, location, and size. Sixty days post-DMBA administration, 80% of the rats developed mammary tumors, and only these animals were included in subsequent studies. All animals were humanely euthanized upon study completion.

DMBA Dosing and Tumor Induction

Female Sprague-Dawley rats, aged 45 to 55 days and weighing 140±10 g, received 20 mg/kg of DMBA weekly for four weeks. The DMBA was prepared in olive oil and delivered orally by gavage needle. Following DMBA administration, rats were observed daily for any growths, noting their development, location, and size. At 60 days post-administration, 80% of the rats had developed mammary tumors. Rats were sacrificed at the end of the study period.

Collection of blood

Rats were anaesthetized using diethyl ether on cotton and placed in a desiccator. A blood sample of 1 ml was collected through the conjunctiva at the back of the eye through the retro-orbital sinus. After collecting the blood samples, the tube was withdrawn, the eyelids were closed and slight pressure was applied with gauze square was placed to stop the flow of blood. The blood sample was collected for estimation of haematological, biochemical parameters.

Hematological Parameters

Blood samples were collected in 1% EDTA vials to estimate Haemoglobin using Sahli's method and Packed Cell Volume using the microtome method. The blood haematological analysis was done for estimation of Haemoglobin (Hb), Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Platelets Count, Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC).

Blood biochemical parameters

The blood sample was collected for separate serum to perform biochemical estimation using Auto analyser with commercial

reagent kits. The blood biochemical analysis was done for estimation of Total protein (TP), Serum albumin (A), Serum Globulin (G), Serum Albumin/Globulin ratio (A/G ratio), Serum alanine aminotransferase (ALT), Serum aspartate aminotransferase (AST), Serum Glucose/Blood Glucose (BG) and Serum creatinine (Cr).

Results

Hemoglobin (Hb) levels

The mean Hb levels in g/dL were 15.51±0.21, 11.34±0.23 in the groups I, II, respectively Where the Group II which was treated with only DMBA had shown significant decrease in Hb levels as 11.34±0.23 g/dL and was much lower than those in the control group (15.51±0.21 g/dL). This suggests DMBA-induced anaemia. Al-Asady *et al*. (2020) [1] reported that DMBA produces reactive oxygen species (ROS), which causes oxidative damage and haematological changes such as leucocytosis and anaemia, these findings are in consistent with present findings.

Total Erythrocyte Count (TEC)

The mean TEC levels (× 10^6/µL) were 8.07±0.27, 4.30±0.16 in the Groups I, II, respectively. Group II, treated with DMBA, showed significant decrease in TEC levels (4.30±0.16 × 10^6/µL), which was significantly lower than those observed in the control group (8.07±0.27 × 10^6/µL). This reduction in TEC further supports DMBA-induced anemia, as DMBA is known to cause oxidative stress and damage to erythrocytes. Similar findings were reported by Kumar *et al*. (2021) [9], who demonstrated that DMBA-induced oxidative stress leads to a significant reduction in erythrocyte count.

Total Leukocyte Count (TLC)

The mean TLC levels (× 10^3/µL) were 15.95±0.42, 19.57±0.38 in the Groups I, II, respectively. Group II, treated with DMBA, exhibited the highest TLC levels (19.57±0.38 × 10^3/µL), indicating leukocytosis, a common response to oxidative stress and inflammation induced by DMBA. This finding aligns with the observations of Al-Asady *et al*. (2020) [1], who reported that DMBA generates reactive oxygen species (ROS), leading to inflammatory responses and elevated leukocyte counts.

Platelet Count

The mean platelet counts (× 10^5/µL) were 7.76±0.13, 3.05±0.09, in the Groups I, II, respectively. Group II, treated with DMBA, showed significant decrease in platelet count which was lowest of all groups (3.05±0.09 × 10^5/µL), indicating thrombocytopenia, which is consistent with DMBA-induced hematological toxicity. The findings are supported by Verma *et al*. (2022), who reported that flavonoids like hesperidin can restore platelet counts in chemically induced hematological damage.

Packed Cell Volume (PCV)

The mean PCV levels (%) were 44.59±0.82, 33.88±0.17in the Groups I, II, respectively. Group II, treated with DMBA, showed significant decrease in the PCV levels which was lowest among all groups (33.88±0.17%), indicating a reduction in red blood cell mass, which is because of anemia induced due to DMBA. The results are in line with the findings of Prakash *et al*. (2023) [14], who demonstrated that herbal therapies can normalize PCV levels in DMBA-treated animals.

Mean Corpuscular Volume (MCV)

The mean MCV levels (fL) were 54.45±1.00, 41.00±0.46, in the Groups I, II, III, IV, V, and VI, respectively. Group II, treated with DMBA, showed significant decrease in MCV levels (41.00±0.46 fL), indicating microcytosis, which is due to DMBA-induced anemia. The findings are supported by Urkude *et al*. (2023) [23], who reported that flavonoids can restore MCV levels in chemically induced hematological damage.

Mean Corpuscular Hemoglobin Concentration (MCHC)

The mean MCHC levels (g/dL) were 33.81±0.54, 28.99±0.20, in the Groups I, II, respectively. Group II, treated with DMBA, showed significant decrease in MCHC levels which was lowest (28.99±0.20 g/dL), indicating hypochromia, which is due to DMBA-induced anemia. The results are in line with the findings of Prakash *et al*. (2023) [14].

Mean Corpuscular Hemoglobin (MCH)

The mean MCH levels (pg) were 17.96±0.53, 14.30±0.20, in the Groups I, II, respectively Group II, treated with DMBA, showed significant decrease in MCH levels (14.30±0.20 pg), indicating reduced hemoglobin content per erythrocyte, which is the effect of DMBA-induced anemia. The findings are supported by Urkude *et al*. (2023) [23].

Biochemical Parameters

Blood from all animals was collected by retro-orbital technique and serum was extracted from blood by centrifugation at 2500 rpm for 15 minutes. The effects of DMBA-induced alterations in biochemical parameters was evaluated using biochemical markers such as total protein, serum albumin, globulin, liver function enzymes (SGOT, SGPT), Blood glucose and serum creatinine in the animals.

Total Protein

The mean total protein levels (g/dL) were 6.17±0.29, 4.76±0.34, in the Groups I, II, respectively. The values of total protein in group II (4.76±0.34 g/dL) found to be significantly lowered as compared to Group I. Group II, treated with DMBA, showed significant decrease in total protein levels (4.76±0.34 g/dL), indicating significant hepatic dysfunction and impaired protein synthesis. This aligns with findings by Salem *et al*. (2004) [16], who reported that DMBA-induced oxidative stress disrupts liver function, leading to reduced protein production.

Serum Albumin

The mean serum albumin levels (g/dL) were 3.43±0.24, 1.78±0.31, in the Groups I, II, respectively. Group II, treated with DMBA, exhibited significant decrease in albumin levels (1.78±0.31 g/dL), reflecting severe liver damage and impaired albumin synthesis. These results are in consistent with the findings of Ozdemir *et al*. (2007) [13], who noted that DMBA-induced oxidative stress compromises hepatic albumin production.

Serum Globulin

The mean serum globulin levels (g/dL) were 2.74±0.32, 2.98±0.32, in the Groups I, II, respectively. There was significant difference observed in each group in respect of serum globulin. Group II, treated with DMBA, showed elevated globulin levels (2.98±0.32 g/dL), indicating an inflammatory response and immune activation. This aligns with the findings of Salem *et al*. (2004) [16], who reported that

DMBA-induced toxicity triggers immune system activation, leading to increased globulin production.

SGOT (AST)

The mean SGOT levels (U/L) were 309.76±41.96, 199.34±17.48, in the Groups I, II, respectively. Group II, treated with DMBA, showed significant increase in SGOT levels (199.34±17.48 U/L), indicating hepatocellular damage. This is consistent with findings by Suzuki *et al*. (2003) [17, 18], who reported that DMBA-induced oxidative stress causes liver cell injury, leading to increased SGOT levels.

SGPT (ALT)

The mean SGPT levels (U/L) were 62.38±3.43, 101.33±2.70, in the Groups I, II, respectively. Group II, treated with DMBA, showed the highest SGPT levels (101.33±2.70 U/L), indicating severe liver injury. Prakash *et al*. (2023) [14] also found that *Mangifera indica* extract significantly lowered SGPT levels in DMBA-treated rats, reinforcing the potential of phytochemicals in protecting against hepatic damage. Furthermore, Purankar *et al*. (2024) [11] demonstrated that *Neolamarckia cadamba* significantly reduced SGPT levels in DMBA-induced toxicity models, highlighting the hepatoprotective efficacy of herbal treatments.

Serum Creatinine

The mean serum creatinine levels (mg/dL) were 0.56±0.05, 0.97±0.18, in the Groups I, II, respectively. Group II, treated with DMBA, showed the highest creatinine levels (0.97±0.18 mg/dL), indicating renal dysfunction. Creatinine levels were significantly higher in Group II then other groups. Karhale *et al*. (2024) [8] also found the similar results in their study.

Table 1: Results of mean Hb, TEC, TLC, PCV, MCV, MCH and MCHC in different groups

Parameters\Group	I	II
Hb (g/dL)	15.51 ± 0.21^{b}	11.34 ± 0.23^{a}
TEC (× 10^{6}/µL)	8.07 ± 0.27^{b}	4.30 ± 0.16^{a}
TLC (× 10^{3}/µL)	15.95 ± 0.42^{a}	19.57 ± 0.38^{c}
Platelet (× 10^{5}/µL)	7.76 ± 0.13^{b}	3.05 ± 0.09^{a}
PCV (%)	44.59 ± 0.82^{b}	33.88 ± 0.17^{a}
MCV (fL)	54.45 ± 1.00^{b}	41.00 ± 0.46^{a}
MCHC (g/dL)	33.81 ± 0.54^{b}	28.99 ± 0.20^{a}
MCH (pg)	17.96 ± 0.53^{b}	14.30 ± 0.20^{a}

Note: Superscripts are to be read row wise for mean comparison. Mean bearing similar superscripts in a row do not differ significantly. Mean bearing different superscripts in a row differ significantly at $p<0.05$

Table 2: Results of mean Total Protien, Serum Albumin, Serum Globulin, Albumin: Globulin, SGOT, SGPT, Serum Creatinine and Serum Glucose in different groups

Parameters\ Groups	I	II
Total Protien (g/dL)	6.17 ± 0.29^{b}	4.76 ± 0.34^{a}
Serum Albumin(g/dL)	3.43 ± 0.24^{b}	1.78 ± 0.31^{a}
Serum Globulin(g/dL)	2.74 ± 0.32^{a}	2.98 ± 0.32^{a}
Albumin: Globulin N.S.	1.41 ± 0.22^{b}	0.68 ± 0.16^{a}
SGOT (U/L)	309.76 ± 41.96^{b}	199.34 ± 17.48^{a}
SGPT (U/L)	62.38 ± 3.43^{a}	101.33 ± 2.70^{b}
Serum Creatinine (mg/dL)	0.56 ± 0.05^{a}	0.97 ± 0.18^{b}
Serum Glucose (mg/dL)	88.50 ± 0.86^{a}	145.13 ± 1.81^{b}

Note: Superscripts are to be read row-wise for mean comparison. Means bearing similar superscripts in a row do not differ significantly. Means bearing different superscripts in a row differ significantly at $p<0.05$.

Serum Glucose

The mean serum glucose levels (mg/dL) were 88.50±0.86, 145.13±1.81, in the Groups I, II, respectively. Group II, treated with DMBA, showed the highest glucose levels (145.13±1.81 mg/dL), indicating impaired glucose metabolism. Blood glucose levels were significantly higher in Group II then other groups.

Conclusions

1. Being a carcinogenic agent, DMBA causes oxidative damage, manifesting severe kidney and liver damage.
2. Before planning research with DMBA, careful dose adjustment should be considered to avoid experiment failure and damage to operator.

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Conflict of Interests

Authors declares that, there is no conflict of interest

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