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Original Article

Ameliorative Effects of *Operculina turpethum* and its Isolated Stigma-5,22dien-3-o-β-D-glucopyranoside on the Hematological Parameters of Male Mice Exposed to *N*-Nitrosodimethylamine, a Potent Carcinogen

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ABSTRACT

Objectives: Enormous propensity of plants to synthesize a variety of structurally diverse bioactive compounds, has made the plant kingdom a potential source of chemical constituents with various therapeutic values, including antitumor and cytotoxic activities. Blood is a good indicator to determine the physiological and pathological status of man and animal. The objective of the present study is to determine the effect of *Operculina turpethum* root extract and its isolated glycoside treatment on the hematological parameters in the mice with *N*-Nitrosodimethylamine (NDMA) induced cancer. **Materials and Methods:** The body weights of the animals were recorded before and after the experiment. Non-coagulated blood was tested for total erythrocyte count, total leukocyte count, hemoglobin, differential leukocyte count (DLC) and for other blood indices. **Results:** A significant ($P < 0.01$), ($P < 0.001$) recovery of the red blood cell and white blood cell counts, packed cell volume and hemoglobin content in the host after 21 day treatment was shown. **Conclusion:** These results show that the extract of *Operculina turpethum* is relatively safe following oral administration and have possible stimulatory effect on red blood cell production and there was dose dependent therapeutic effect.

Key words: Blood, haematology, *N*-Nitrosodimethylamine, *Operculina turpethum*

INTRODUCTION

Hematopathology is not only the study of the blood and bone marrow, but also of the organs and tissues which employ blood cells as principal effectors of their physiologic functions. Such would include the lymph nodes, spleen, thymus, and the many foci of lymphoid tissue found along the aero-digestive tract. The reliable criteria for judging the value of any anticancer drug are prolongation of lifespan and decrease of white blood cells (WBC) from blood.[1,2] Hence, a major portion of the current pharmacological research is involved with the anticancer drug design customized to fit new molecular targets.[3] Assessment of hematological profile becomes a pre-requisite to understand the normal functioning of the system and to further confirm the toxic or protective nature of the administered drug or the plant extract.

Blood is a specialized body fluid that delivers necessary substances to the body's cells such as nutrients and oxygen and transports waste products away from those same cells.[4] Blood is composed of plasma and several kinds of cells; these formed elements of the blood are erythrocytes,

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leucocytes and thrombocytes.[5] Hematological analyzes, which include packed cell volume (PCV), red blood cells (RBCs), total leukocyte counts (TECs), and differential counts, provide information about the hematopoietic system and immunological responses. The mean corpuscular values (MCV), mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) computed from the RBC, PCV, and HBC are usually useful in elucidating and classifying blood condition morphologically; they represent an estimation of the alterations in size and hemoglobin content of individual red blood cells.[6]

Operculina turpethum which is also known as Indian Jalap is a convolvulaceous plant which is found throughout India, China, Ceylon, Australia, and is occasionally cultivated in botanical gardens as an ornamental plant. There are two varieties, viz., *Sveta* or white turpeth, and *Kirshna* or black turpeth[7] *Operculina turpethum*, which is commonly known as trivit, is a large stout perennial twinner with milky juice and fleshy branched roots. It is one of the plants mentioned in the literature having claims of activity against liver disorders and cancer.[8] It also has anthelmintic expectorant, antipyretic, anti-inflammatory and purgative properties. It contains a wide variety of phyto constituents, which are useful in treatment of different ailments and includes glycosidic resin, coumarins, beta-sitosterol, and essential oil.[9]

In recent years, one of the areas which attracted a great deal of attention is the possible therapeutic potential of antioxidants in controlling degenerative diseases associated with marked oxidative damage. The pathophysiology of various clinical disorders, including ischemia, reperfusion injury, atherosclerosis, acute hypertension, hemorrhagic shock, diabetes mellitus and cancer have been implicated to reactive-oxygen species (ROS). Several phytochemicals have been isolated from the plants and assessed for their antioxidant potential. Steroidal glycoside isolated from the roots of *Operculina turpethum* belongs to the class of phytosterols, which are shown to possess cholesterol lowering,[10] immune-modulating as well as anticancer property.[11-14] Cancer chemoprevention has been defined as a process facilitated by blocking induction of neoplastic process or preventing transformed cells from progression to malignant phenotypes by administration of one or more chemical entities, either as synthetic drugs or naturally occurring phytoconstitutents. In cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia.[15,16] The anemia encountered in cancer bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency order to hemolytic or myelopathic conditions.

Iron is an essential component of many enzymes in cells and is also part of the heme group in hemoglobin (which consists of a porphyrin ring containing iron). Much of the body's iron stores are within red blood cells where iron is critical for hemoglobin synthesis. The MCV is an index of the size of the RBCs. When the MCV is below normal, the RBCs will be smaller than normal and are described as microcytic. When the MCV is elevated, the RBCs will be larger than normal and are termed macrocytic. RBCs of normal size are termed normocytic.[17]

N-Nitrosodimethylamine (NDMA), is known to cause perturbations in the nuclear enzymes involved in deoxyribonucleic acid (DNA) repair/replication and is normally used as a carcinogen to induce liver cancer in animal models. Chemical induced liver injury depends mostly on the oxidative stress in hepatic tissue and underlies the pathology of numerous diseases, including cancer. Experimental, clinical and epidemiological studies have provided evidences supporting the role of reactive oxygen species in the etiology of cancer. *N*-Nitrosodimethylamine (NDMA) is a member of a family of extremely potent carcinogens, the *N*-nitrosamines[18] [Figure 1].

N-Nitrosodimethylamine, can occur in drinking water through the degradation of dimethylhydrazine, a component of rocket fuel, as well as from several other industrial processes. It is also a contaminant of certain pesticides and found as disinfection by-product in waste water treatment plants. So the chances of NDMA reaching human biological system are very high.[19] In the present study, the effect of *Operculina turpethum* and its isolated Stigma-5,22dien-3-O-β-D-glucopyranoside on the hematological and serological parameters of mice bearing cancer was evaluated.

EXPERIMENTAL METHODOLOGY

Chemicals

TBA, TCA, HCl, pyrogallol, H_2O_2, triton-x, BSA, copper sulfate, ascorbic acid, thiourea etc., All chemicals used in the study were of analytical reagent grade and were purchased from reliable firms (SRL (India), MERCK, RANBAXY, HIMEDIA, TRANSASIA). NDMA was purchased from SIGMA.

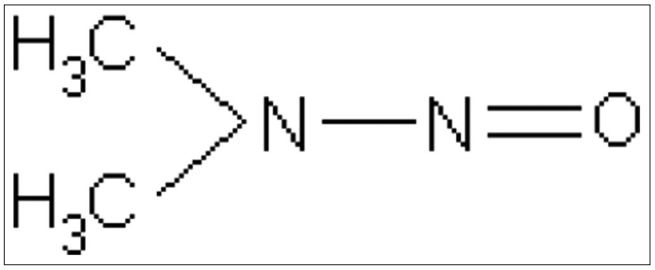


Figure 1: Structure of *N-Nitrosodimethylamine*

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Animal care and monitoring

Healthy male Swiss albino mice (*Mus musculus*) (4-6 months old, weighing 20-30 g) were procured from C.C.S. Haryana Agricultural University (Hisar, India). They were housed under standard laboratory conditions of light (12:12 h L: D cycle), temperature (23 ± 2°C) and relative humidity (55 ± 5%). Animals lead free access to standard food pellet diet (Hindustan Lever Limited: metal contents in parts per million dry weight: Cu 10.0, Zn 45.0, Mn 55.0, Co 5.0, Fe 75.0) and drinking water *ad libitum* throughout the study.

Plant material

Operculina turpethum was collected from Pharmacological garden of CCSHAU Hisar, Haryana, India in the month of November 2012. The plant was identified with the help of available literature and authenticated by Botanist of Krishi Vigyan Kendra Rohtak, Haryana, India.

Preparation of ethanolic extract

The freshly collected *Operculina turpethum* roots were dried in shade and coarse powder was extracted. Dried powdered material was placed in the Soxhlet thimble with ethanol in 500 ml flat bottom flask. Further refluxed for 18 h at 80°C for two days. Collected solvent was cooled and poured in a glass plate. The filtrate was dried in hot air oven below 50°C for 48 h and kept in dissector for 2 days. The yield of the extract was 12.5% w/w of powdered plant material for further exploration. Collected dried extract was stored at 5°C in air-tight containers.

Isolation and characterisation of stigma-5,22dien-3-o-β-D-glucopyranoside (Isolated Glycoside; IG)

Isolation of IG was achieved by TLC, Column Chromatography and HPLC whereas the characterization was achieved by IR, NMR and LCMS. The nomenclature of the IG was achieved and it was then assessed for its anti-carcinogenic properties through various hematological parameters.

Ethical clearance

The animal experiments were carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Institutional Animal Ethics Committee approved experimental design performed in this study for the use of Swiss Albino mice as an animal model for the study.

Acute oral toxicity studies (LD50)

The acute toxicity of the plant extract was evaluated in mice (six per group) by preparing five different doses (100, 500, 1000, 1500 and 2000 mg/kg) and administered orally using gavages. Animals were kept without food for 18 h prior to dosing and were monitored continuously for 3 days after dosing for any sign of toxicity. The LD50 value of the extract was calculated arithmetically using the method described by Hamilton.[20]

$$LD50 = \text{Lethal dose} - \Sigma (a \times b)/N$$

where a is the dose difference, b is the mean mortality and N is the number of animals in each group.

Treatment regime

Adult Swiss albino male mice divided into ten groups of 6 mice each group were treated by oral gavage. Treatment consisted of simultaneous dosing of NDMA (*N*-Nitrosodimethylamine, 10mg/kg b.wt.) followed by OTE (*Operculina turpethum* extract). The animals were then euthanized 21 days after NDMA administration. NDMA was given on three consecutive days of each week for three successive weeks along with the plant extract.

The groups were as follows
Group 1 - Control
Group 2 - NDMA treated (10 mg/kg body weight)
Group 3 - NDMA + OTE (300mg/kg body weight)
Group 4 - NDMA + OTE (400mg/kg body weight)
Group 5 - OTE (300 mg/kg body weight)
Group 6 - OTE (400mg/kg body weight)
Group 7 - NDMA + Standard antioxidant (BHA1%)
Group 8 - BHA (Butylated Hydroxy Toluene) (1%)
Group 9 - IG (Isolated Glycoside; 50 mg/kg body weight)
Group 10 - NDMA + IG (Isolated Glycoside; 50 mg/kg body weight)

The doses of the plant extract, NDMA and standard were decided on the basis of previously published reports.[21]

Body weight

The body weight of the animals was calculated before and after the experiment and is expressed in grams. The change in the body weight was calculated according to the following formula.

$$\text{Change in the body weight \%} = \frac{\text{Change in the body weight}}{\text{Initial body weight}} \times 100$$

Collection of blood

Twenty first day after the start of the experiment, the animals were procured and the blood was collected by puncturing the retro-orbital plexus from the eye in the EDTA-vials and stored in the refrigerator for analyzes.

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Determination of hematological parameters

Total erythrocyte count and total leukocyte count were done by method given by Berkson *et al.*[22] Hemoglobin estimation was performed using Haden's method.[23] Hematocrit (PCV) was calculated by Wintrob method.[24] Platelet count was performed by method of Dorland.[25] Erythrocyte indices MCV, MCHC, and MCHC were determined from values obtained from RBC count, hemoglobin concentration and PCV values.[26]

Statistical analysis

Data are expressed as the mean ± SEM. The data was analyzed by analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS. 11). The results were evaluated at a significance level of $P < 0.01$ and 0.001 which were considered significant and highly significant, respectively.

RESULTS

Isolation and Characterisation of Stigma-5,22dien-3-o-β-D-glucopyranoside from the roots of Operculina turpethum

The isolated compound was found to be a steroidal glycoside.

Acute toxicity study

The result of the toxicity test of *Operculina turpethum* extract for 3 days did not show any clinical adverse effect of substance-related toxicity on the animals, such as restlessness, hematuria, diarrhea and muscle-coordinated movement. Similarly, there was no mortality or morbidity observed at any tested doses except at the 2000 mg/kg dose. The LD50 value of the extract was found to be 1917.66 mg/kg.

Effect on the body weight

In the present study, there was a drastical change in the body weight of animals of group II which is a sensitive indicator that the compound NDMA has caused toxicity leading to reduction in the body weight. On the other hand the loss in the weight is significantly recovered in animals treated with the plant extract. Gross pathological observation of the organs showed no gross abnormalities in the morphologies, features, consistencies and appearances of the liver, kidney, heart, spleen, lungs and testes of the male mice treated for 21 days with the extract and only the changes in the weight were observed.

It is evident from the results that there is not much change in the weight of animals in control group with time. The initial average body weight of the mice was (25.5 ± 0.439) Control, (26.0 ± 0.248) NDMA, (25.7 ± 0.078) NDMA + OTE 300 mg/kg body weight, (25.8 ± 0.598) NDMA + OTE 400 mg/kg, (25.0 ± 0.414) OTE 300 mg/kg body weight, (25.6 ± 0.292) OTE 400 mg/kg body weight, (25.8 ± 0.131) NDMA + BHA,(25.6 ± 0.144) BHA, (25.88 ± 0.665) IG and (25.6 ± 0.427) NDMA + IG. The weight of the animals after the completion of the experiment were significantly reduced (25.2 ± 0.598) Control, (21.3 ± 0.505) NDMA, (24.2 ± 0.086) NDMA + OTE 300 mg/kg body weight, (25.8 ± 0.331) NDMA + OTE 400 mg/kg body weight, (24.7 ± 0.182) OTE 300mg/kg body weight, (24.5 ± 0.439) OTE 400 mg/kg body weight, (24.2 ± 0.057) NDMA + BHA and increased as (27 ± 0.373) BHA, whereas the animals treated with the isolated compound showed the weight as (25.52 ± 0.589) IG and (24.6 ± 0.268) NDMA + IG, respectively [Table 1]. Decrease in the body weight was observed throughout the study duration in almost all the groups except group VIII. This deduction in the body weight can be attributed to the fact that the compound NDMA caused a significant toxicity in mice. There is a significant increase in the body weight in group VIII, which can be attributed to the antioxidant effect of BHA. The harmful effect caused by the NDMA is significantly recovered by the administration of the OTE at the concentration of 300 mg/kg b.wt and at the concentration of 400 mg/kg b.wt., respectively.

Hematological parameters

Hematological parameters [Table 2] of mice on day 21were found to be significantly altered compared to the normal group. In the animal group given with NDMA, the average values ($P > 0.001$) of RBC count was lesser after 21 days,

Table 1: Analysis of the body weight of mice in different groups before and after treatment

Groups	Dose	Initial weight (gm)	Final weight (gm)	% Change
I	Control	25.5±0.439	25.2±0.598*	1.17
II	NDMA	26.0±0.248	21.3±0.505$^{\alpha}$*	18.07
III	NDMA+OTE (300 mg/kg b.wt.)	25.7±0.078	24.2±0.086$^{\alpha}$	5.83
IV	NDMA+OTE (400 mg/kg b.wt.)	25.8±0.598	25.8±0.331*	7.74
V	OTE (300 mg/kg b.wt.)	25.0±0.414	24.7±0.182$^{\alpha}$*	5.22
VI	OTE (400 mg/kg b.wt.)	25.6±0.292	24.5±0.439$^{\alpha}$*	4.55
VII	NDMA+BHA	25.8±0.131	24.2±0.057$^{\alpha}$*	6.31
VIII	BHA (1%)	25.6±0.144	27±0.373$^{\alpha}$*	5.18
IX	IG (50 mg/kg)	25.88±0.665	25.52±0.589$^{\alpha}$	1.39
X	NDMA+IG (10+50 mg/kg)	25.6±0.427	24.6±0.268*	3.90

Values are expressed as mean±S.E.M for six mice in each group. Control group $^{\alpha}P<0.01$, *$P<0.001$ vs. treated (NDMA) group. NDMA = N-Nitrosodimethylamine, OTE = Operculina turpethum extract, BHA = Butylated hydroxy toluene, IG = Isolated glycoside

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when compared to control group. Administration of low dose and high dose of the plant extract and the isolated compound to NDMA treated animals in groups showed improved RBC count as compared to NDMA group. The total WBC count was found to be increased with a reduction of Hb content of RBC in the NDMA treated animals.

In case of PCV, NDMA exposure produced a significant decline in PCV of mice as compared to control group. The mean PCV in control group was 37.813 ± 0.469%, whereas those of low and high dose plant extract groups and NDMA were 37.513 ± 0.761%, 37.394 ± 0.722% and 33.294 ± 0.747%, respectively. The mean PCV of the low and high dose plant group and NDMA were significantly different from that of control group ($P < 0.001$). Also, the IG treatment on animals showed the PCV of 37.312 ± 0.561% significantly ($P < 0.001$) different from the control whereas in NDMA + IG, the PCV was observed to be 37.149 ± 0.611 ($P < 0.001$), different from the NDMA treated group [Table 2].

Reduction in platelets count in experimental animals has been reported to indicate adverse effect on the oxygen-carrying capacity of the blood as well as thrombopoietin, as it was observed in NDMA group.[27,28] Results from this study show that the platelet count was unaltered signifying that the oxygen carrying capacity of the blood was unaffected when the animal were administered with IG at a dose of 50 mg/kg.

White blood cell differentials are indicators of the ability of an organism to eliminate infection. An increase in the number of circulating leukocytes is rarely due to an increase in different types of leukocyte. Neutrophils attack and destroy bacteria in the blood.[29] The reduced neutrophils will adversely affect the phagocytosis activity in the animals. Lymphocytes are the main effector cells of the immune system. This reduced neutrophil count caused by NDMA probably indicates that the ability of the body to attack and destroy invading bacteria, viruses and other injurious agents (phagocytosis) was compromised. However, *Operculina turpethum* at tested doses of 300 and 400 mg/kg b.wt. produced a significantly increased ($P < 0.01$) blood levels of monocytes and neutrophils count when compared to NDMA group [Table 3].

The increased MCV in the NDMA treated group can be as a result of macrocytosis. In conditions of regenerative anaemia, microcytosis have been described.[30,31] The increase in MCV could be as a result of larger subpopulation

Table 2: Hematological parameters of mice given with different treatments in groups

Group	Parameters	Total RBC (106/uL)	Total WBC (103/uL)	Hemoglobin (g/dL)	PCV (%)	Platelet count (103/uL)
I	Control	7.571±0.743*	18.210±0.422*	12.538±0.72*	37.813±0.46*	235.180±0.060*
II	NDMA (10 mg/kg)	4.252±0.877$^{\alpha}$	22.419±0.614$^{\alpha}$	8.311±0.77$^{\alpha}$	33.294±0.74$^{\alpha}$	198.544±0.622$^{\alpha}$
III	NDMA+OTE (10+300 mg/kg)	5.057±0.874$^{\alpha}$*	20.910±0.307$^{\alpha}$*	9.337±0.78$^{\alpha}$*	33.867±0.67$^{\alpha}$*	208.671±0.541$^{\alpha}$*
IV	NDMA+OTE (10+400 mg/kg)	5.691±0.776$^{\alpha}$*	19.776±0.510$^{\alpha}$*	9.581±0.44$^{\alpha}$*	34.950±0.76$^{\alpha}$*	217.521±0.148$^{\alpha}$*
V	OTE (300 mg/kg)	7.372±0.637$^{\alpha}$	18.479±0.732$^{\alpha}$	12.326±0.71$^{\alpha}$	37.513±0.76$^{\alpha}$	233.613±0.567$^{\alpha}$
VI	OTE (400 mg/kg)	7.344±0.874$^{\alpha}$	17.798±0.603$^{\alpha}$	12.480±0.64$^{\alpha}$	37.394±0.72$^{\alpha}$	234.521±0.693$^{\alpha}$
VII	NDMA+BHA (10+1%)	6.132±0.518 $^{\alpha}$*	19.988±0.422$^{\alpha}$*	10.037±0.70$^{\alpha}$*	37.006±0.45$^{\alpha}$*	220.500±0.855$^{\alpha}$*
VIII	BHA (1%)	7.666±0.671$^{\alpha}$	18.569±0.555$^{\alpha}$	12.411±0.67$^{\alpha}$	37.630±0.75$^{\alpha}$	236.159±0.915$^{\alpha}$
IX	IG (50 mg/kg)	7.192±0.775$^{\alpha}$	18.522±0.601$^{\alpha}$	12.551±0.71$^{\alpha}$	37.312±0.56$^{\alpha}$	235.443±0.734$^{\alpha}$
X	NDMA+IG (10+50 mg/kg)	6.096±0.906$^{\alpha}$*	18.964±0.578$^{\alpha}$*	10.148±0.69$^{\alpha}$*	37.149±0.61α*	221.026±0.687$^{\alpha}$*

Values are expressed as mean±S.E.M for six mice in each group. Control group $^{\alpha}P<0.001$, $^{*}P<0.001$ vs. treated (NDMA) group. NDMA = N-Nitrosodimethylamine, OTE = *Operculina turpethum* extract, BHA = Butylated hydroxy toluene, IG = Isolated glycoside, RBC = Red blood cells, WBC = White blood cells, PCV = Packed cell volume

Table 3: Stimulating effect of *Operculina turpethum* and its isolated glycoside on the Differential leucocyte count of treated animals

Group	Parameters	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)
I	Control	62.565±0.768*	13.532±0.828*	6.546±0.690*
II	NDMA (10 mg/kg)	68.478±0.815$^{\alpha}$	9.408±0.776$^{\alpha}$	4.429±0.755$^{\alpha}$
III	NDMA+OTE (10+300 mg/kg)	65.289±0.931$^{\alpha}$*	10.455±0.840$^{\alpha}$*	5.289±0.929$^{\alpha}$*
IV	NDMA+OTE (10+400 mg/kg)	65.161±0.365$^{\alpha}$*	10.831±0.846$^{\alpha}$*	5.713±0.730$^{\alpha}$*
V	OTE (300 mg/kg)	68.285±0.949$^{\alpha}$	13.223±0.798$^{\alpha}$	6.220±0.856$^{\alpha}$
VI	OTE (400 mg/kg)	68.348±0.679$^{\alpha}$	13.268±0.814$^{\alpha}$	6.331±0.913$^{\alpha}$
VII	NDMA+BHA (10+1%)	65.036±0.505$^{\alpha}$*	11.544±0.612$^{\alpha}$*	6.045±0.964$^{\alpha}$*
VIII	BHA (1%)	62.923±0.369$^{\alpha}$	13.598±0.854$^{\alpha}$	6.375±0.844$^{\alpha}$
IX	IG (50 mg/kg)	62.476±0.759$^{\alpha}$	13.496±0.758$^{\alpha}$	6.329±0.662$^{\alpha}$
X	NDMA+IG (10+50 mg/kg)	65.107±0.659$^{\alpha}$*	11.465±0.860$^{\alpha}$*	6.057±0.923$^{\alpha}$*

Values are expressed as mean±S.E.M for six mice in each group. Control group $^{\alpha}P<0.001$, $^{*}P<0.001$ vs. treated (NDMA) group. NDMA = N-Nitrosodimethylamine, OTE = *Operculina turpethum* extract, BHA = Butylated Hydroxy Toluene, IG = Isolated Glycoside

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of erythrocytes with increased red cell distribution width (RDW). RDW is an electronically calculated parameter of the RBC which indicates the presence of an increased number of RBC subpopulation: larger, smaller, or a combination with a shift to the right indicating a subpopulation of larger RBCs [Table 4].

Moreover, the effect of *Operculina turpethum* root extract on RBC, total WBC and Platelet counts, Differential leucocyte count was dose dependent. This dose-dependence phenomenon also explains the effect of glycoside on hematopoietic system in such a way that the higher dose of the extract showed better effects than low dose. Treatments brought back the hemoglobin content, RBC and WBC count more or less to normal levels and this indicates that *Operculina turpethum* and its isolated compound possess protective stimulating action on the hematopoietic system.

DISCUSSION

Extracts of plants have been used as traditional remedies for the treatment of many disorders in all over the world. Several phyto-chemicals associated with therapeutic properties have been isolated from the plant extract and have been evaluated for their protective and therapeutic action. In the present study, one such phytochemical, Stigma-5, 22dien-3-O-β-D-glucopyranoside, from the plant *Operculina turpethum*, was isolated and evaluated for its effect on the blood indices of male mice. Alteration in weight is an indication of impairment in the normal functioning of the organs. An increase in organ-body weight ratio is an indication of inflammation while a decrease may be due to cell constriction.[32] The intoxication of NDMA produced some deleterious effects in the animals which lead to a decrease in the body weight of animals treated with NDMA which was further recovered comparably with the control animals by the treatment with extract and the isolated glycoside, indicating the therapeutic ability of the plant *Operculina turpethum*.

Bone marrow is responsible for the production of red blood cells, white blood cells and platelets. Reduced level of total erythrocyte count (TEC) was observed in NDMA treated mice. The mechanism of action by which NDMA aggravated pathogenesis of anemia could involve down regulation of erythropoietin activity. Decreased level of RBC has been contributed to reduction of erythropoisis in bone marrow and showed rapid rate of destruction of peripheral RBC in spleen. In the animals treated with just the plant alone, there was no significant destruction of red blood cells and no significant change in the rate of production of RBC (erythropoiesis), suggesting the non-toxic nature of the plant extract to red blood cells. Diminished erythropoiesis, shortened red cell life span, hemorrhage, hemodilution, have been considered either singly or in combination for the etiology of cancer.[33] Decreased level of Hb can be related with reduced size of RBC, impaired biosynthesis of heme in bone marrow or due to reduction in rate of formation of erythrocyte. In cancer therapy also low blood cell counts is one of the common side effects caused by radiation therapy and chemotherapy. Complications like myelosuppression and thrombocytopenia are usually associated during cancer chemotherapy.[15]

Platelets are the blood cells involved in coagulation.[34] Coagulation of blood requires that the platelets should be in sufficient size, number and function. It has been pointed out, that reduced blood platelets affect the viscosity of blood, which is correlated positively to blood pressure.[35] Platelet aggregation plays a pivotal role in the physiopathology of thrombotic diseases. Moreover, platelet activity may play a major role in the development

Table 4: Therapeutic effect of *Operculina turpethum* and its isolated glycoside on the blood indices of different treatment groups

Groups	Parameters	MCV (fL)	MCH (pg)	MCHC (g/dL)
I	Control	49.996±0.004*	16.571±0.494*	0.332±0.990*
II	NDMA (10 mg/kg)	78.353±0.004$^{\alpha}$	19.565±0.229$^{\alpha}$	0.250±0.994$^{\alpha}$
III	NDMA+OTE (10+300 mg/kg)	67.003±0.005$^{\alpha}$*	18.474±0.334$^{\alpha}$*	0.276±0.995$^{\alpha}$*
IV	NDMA+OTE (10+400 mg/kg)	61.476±0.001$^{\alpha}$*	16.839±0.227$^{\alpha}$*	0.274±0.983$^{\alpha}$*
V	OTE (300 mg/kg)	50.974±0.001$^{\alpha}$	16.760±0.133$^{\alpha}$	0.329±0.993$^{\alpha}$
VI	OTE (400 mg/kg)	50.927±0.241$^{\alpha}$	17.001±0.350$^{\alpha}$	0.334±0.990$^{\alpha}$
VII	NDMA+BHA (10+1%)	60.661±0.002$^{\alpha}$*	16.432±0.124$^{\alpha}$*	0.271±0.991$^{\alpha}$*
VIII	BHA (1%)	49.145±0.011$^{\alpha}$	16.216±0.212$^{\alpha}$	0.330±0.091$^{\alpha}$
IX	IG (50 mg/kg)	51.899±0.193$^{\alpha}$	17.465±0.304$^{\alpha}$	0.336±0.990$^{\alpha}$
X	NDMA+IG (10+50 mg/kg)	59.780±0.001$^{\alpha}$*	16.648±0.487$^{\alpha}$*	0.273±0.991$^{\alpha}$*

Values are expressed as mean±S.E.M for six mice in each group. Control group $^{\alpha}P<0.001$, *$P<0.001$ vs. treated (NDMA) group. NDMA = N-Nitrosodimethylamine, OTE = *Operculina turpethum* extract, BHA = Butylated hydroxy toluene, IG = Isolated glycoside, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, MCV = Mean corpuscular volume of erythrocytes

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as well as in the stability of atherosclerotic plaques and as a consequence, antiplatelet agents have been used clinically in patients at risk for myocardial ischemia, unstable angina and acute myocardial infarction.[36,37] The number of platelets was significantly ($P < 0.001$) recovered by the treatments with extract and isolated glycoside.

The total white blood cell (WBC) counts and the DLCs reflect the systemic status of an animal in relation to its response to injurious agents, stress, and/or deprivation; the indices are of value in confirming or eliminating a tentative diagnosis, in making a prognosis and guiding therapy. There was a significant increase in DLC count, which mainly consisted of neutrophils and monocytes. The increased level of DLC and percentage of neutrophils and monocytes suggest that NDMA elicited an inflammatory response and cause alteration in bone marrow and function of immune system.

CONCLUSION

In conclusion, no toxic effects were observed on the hematological parameters of mice with different treatments with *Operculina turpethum* extract and its isolated glycoside used in this work whereas the treatment was found to be beneficial for the experimental animals in evoking the immune-stimulatory response. Hence the plant can be further evaluated and used for various pharmacological formulations.

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