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RESEARCH ARTICLE

Evaluation of Anticancer properties of *Taxusbaccata* and Badri cow urine in mice: Clinicohematological study

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Abstract

In the present investigation, the anticancerous effect of *Taxusbacaata* and distilled Badri cow urine was studied in mice for clinicohematology and body weight for a period of six month at an interval of 15 days. The study revealed that the values of total leucocyte count (TLC), absolute lymphocyte count (ALC) and absolute neutrophil count (ANC) were significantly increased in the treated groups of mice either by CUD alone and in combination with*Taxusbaccata* extracts. At180th day, it was found that there was an increase in body weight, Hemoglobin content (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), absolute lymphocyte count (ALC) and absolute neutrophil count (ANC) levels in CUD +A treated group as 23%, 23.99%, 41%, 40%, 40.31%, and 40.13%, respectively. This clearly indicate the, increase in vitality and defence mechanism of body which in turn helps in further healing of cancer.

Introduction

Cancer is a disease involving dynamic changes in genome and is characterized by the uncontrolled, uncoordinated and purposeless proliferation of malignant cells and their ability to spread, either by growth in the adjacent tissue through invasion or by implantation at distant sites through metastasis. World cancer report issued by International Agency for Research on Cancer (IARC) reported in 2003 that cancer rate is set to increase at an alarming rate globally. The 5-year relative survival rate for all cancers diagnosed between 1999-2005 is 68%, up from 50% in 1975-1977. (Kinzler and Vogelstein, 2002)(Jemal et al., 2011).

In India about 70% of population obtains medical help from private practitioners and half of those who seek medicinal help obtain it from alternative and traditional medicine (Kumar et al., 2004). Poverty and socioeconomic status are other hurdles in treatment (Pal and Mittal, 2004). American cancer society defines complementary and alternative medicines (CAM) simply as anything which is not conventional (Zollman and Vickers, 1999; Park *et al.,* 2003). There are various CAM used for cancer patient worldwide viz. Herbal medicine, acupuncture, Ayurveda, biological agents, traditional Chinese medicines, meditation and yoga etc. However, use of herbs for cancer treatment is very popular throughout the world.

Distilled cow urine protects DNA and repairs it rapidly as observed after damage due to pesticides. It protects chromosomal aberrations by mitocycin in human leukocyte. Cow urine helps the lymphocytes to survive and not to commit suicide (apoptosis). Pathogenic effect of free radicals are prevented through cow urine therapy. Use of cow urine one can get the charm of a youth as it prevents the free radicals formation. *Taxus baccata*, commonly known as THUNER, which is mainly found in high altitude area like, Patwadangar, Nainital India also had anticancer and antiviral properties. It is a small to medium-sized evergreen tree, growing 10-20 m tall, exceptionally up to 28 m. It is relatively slow growing, but can be very long-lived, with the maximum recorded trunk diameter of

4 m probably only being reached in around 2,000-4,000 years. Thuner is the oldest plant at high altitude region of Uttarakhand. Most parts of the tree are toxic, except the bright aril surrounding the seed, enabling ingestion and dispersal by birds. The major toxin is the alkaloid taxane. Phytochemical analysis of extracts of leaves and bark showed the presence of lignans, flavonoid, glycosides, sterols, sugar, amino acid, and triterpenoid, alkaloids, steroids, tannins, mucilage, fixed oil, phenolic compounds and protein.The leaves are the principal source of taxol; the anti-cancer drug, but has not been widely exploited in this connection (Hartzell, 2003).

Considering the severity of cancer as a disease of man and animals and complexity of therapeutic approaches and their harmful side effects, it was planned to study the effect of *Taxusbaccata* preparation along with cow urine distillate in mice as measured through clinical haematology

Materials and Methods

1. Extract preparation

Extracts of leaves and bark of *T. baccata* were prepared by applying the standard methods with different solvents like; Aqueous, ethanol, methanol and ether as described by Govindachari*et al*., (1999) and Udupa*et al*., (1995).

In-vivo study

2. Experimental design

Present study was performed in mice maintained in the experimental animal house in Institute of Biotechnology, G.B. Pant University of Agriculture and Technology, Patwadangar, Nainital, Uttarakhand, India. A total of 97 animals were equally divided into 11 groups. The mice were housed in clean polypropylene cages and fed adlibitum with commercially available feed and water. The experiment was carried out in accordance with the Institutional Animal Ethical Committee (IAEC), G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. The 11 groups were:Control group(9 mice), Negativecontrol(DEN treated mice)(8 mice), CUD(without DEN)(8 mice), A (Aqueous extract of leaves of *Taxusbaccata*)(8 mice), (Ethanolic extract of leaves of *Taxusbaccata*)(8 mice), G(Methanolic extract of bark of *Taxusbaccata*)(8 mice), H(Ether extract of Bark of *Taxusbaccata*)(8 mice), CUD(Cow Urine Distillate)(with DEN)(8 mice), CUD+A(8 mice), CUD+B(8 mice), CUD + G(8 mice), CUD+H (8 mice).

Single dose of diethyl nitrosamine (DEN) @ 200 µl/kg body weight was given to each mice of negative control group and tests groups. 500 ml of each extract were made by adding 20% of extract in 500ml of distilled water (Kumar *et al.,* 2004b). The mice of 9 test groups were given different extracts of *taxusbaccata* alone and in combination with CUD (2ml/day/mice), daily p.o., from day 1 for 6 months; however, the mice of negative control group were maintained with routine feed and water.Body weight of mice were taken regularly at an interval of 15 day till the end of the experiment.Total leucocyte count (TLC), absolute neutrophil count (ANC), absolute leucocyte count (ALC), hemoglobin, total erythrocyte count (TEC) and hemoglobin (Hb) content of all the experimental animals in different groups were determined regularly at an interval of 15 day till the end of the experiment as per in standard procedures (Chauhan, 2005).

Results

In-vivo study was carried out in mice using DEN as carcinogen and plant extracts alone and/or combination with CUD as test material for a period six month.

Body Weight

Body weight of mice were taken in gram at an interval of 15 days till the end of experiment. Data of body weight change during experiment were givens in **table-1.** Initially, the mean bodyweight of control was 21.47±1.21 gm and after 6 month the mean body weight of mice was 25.86±1.87 gm. In DEN (negative control) treated group the initial mean body weight at zero day of experiment was 22.73±1.33 gm, which decreased to 19.16±1.81 gm at the end of experiment. But in CUD treated group mean body weight at zero day was 22.43±1.36 gm and at the end of experiment it was 23.91±1.21 gm.CUD treated group in which the carcinogen has been given, the initial mean body weight at zero day was 21.42±1.56 gm. After the end of experiment, the mean body weight was 21.06±1.91 gm. In test group A, the zero day mean body weight 23.13±1.54 gm, which marginally increased at the end of experiment to 23.52±1.01gm.In the group CUD+A the mean in body weight at zero day was 21.36±1.47 gm which was 26.48±0.902 at the end of experiment. In the group CUD+B, the mean body weight was 22.76±1.44 gm at zero day and was 24.80±1.09 gm at the end of experiment. In CUD+G and CUD+H groups, the mean body weight at zero day was 22.97±1.37 gm and 22.81±1.21 gm which was increased to 24.67±0.941 gm and 24.60±1.01 gm at the end of the experiment. In group B, G and H, the mean body weight at zero day was found 22.81±1.26 gm, 22.41±1.32 gm and 22.51±1.28 gm respectively and at the end of experiment the body weight reaches

to 23.40±1.02, 23.33±1.91 and 23.28±1.89, respectively.

Table 1: Body weight in gm of experimental mice at an interval of 15 days (Mean±SE).

Groups/Days	0 Day	15	30	45	60	75	90	105	120	135	150	165	180
Control	21.47±1.21*	22.52±1.32	22.92±1.47	23.17±1.07	23.19±1.36	24.31±1.41	24.56±1.53	25.07±1.43	25.19±0.947	25.37±1.12	25.69±1.49	25.72±0.989	25.86±1.87
CUD (no DEN)	22.43±1.36*	22.48±1.29*	22.51±1.33	22.54±1.42*	22.61±1.62**	22.79±1.46	22.94±1.53	23.08±1.67*	23.48±1.82	23.54±1.29*	23.67±1.68	23.82±1.92	23.91±1.21**
DEN	22.73±1.33**	23.12±1.42	23.49±1.36*	23.79±1.21*	24.01±1.36	24.67±1.17**	24.92±1.07*	25.16±0.941	24.01±1.21*	23.72±1.06	22.09±1.09*	21.87±1.31*	19.16±1.81
CUD	21.42±1.56*	21.62±1.41*	21.71±1.52*	22.07±1.02	22.67±1.17**	22.89±1.19	23.12±1.23*	22.92±1.16	22.66±0.941	22.52±1.12	22.26±1.07	22.12±1.13*	21.06±1.91*
A	23.13±1.54*	23.63±1.71	24.76±1.23	24.93±1.32**	25.11±1.16*	25.72±1.21	25.91±1.47	24.37±1.41**	24.87±1.31	23.85±1.03**	23.71±1.07	23.68±1.13	23.52±1.01*
B	22.81±1.26	23.12±1.21**	23.71±1.28	23.91±1.17	24.11±1.24*	24.52±1.31**	25.03±1.28*	24.76±1.19*	24.10±1.07*	23.81±1.23	23.71±1.16*	23.67±1.09	23.40±1.02*
G	22.41±1.32	22.91±1.61*	23.14±1.10**	23.57±1.17	23.87±1.36*	24.10±1.30	24.47±1.28	24.32±1.21*	24.10±1.07	23.73±1.22*	23.64±0.940	23.50±1.11	23.33±1.91*
H	22.51±1.28*	22.68±1.31	22.91±1.12*	23.41±1.21**	23.62±1.31	23.82±1.40*	23.98±1.33	23.90±1.21	23.81±1.17	23.63±1.07	23.48±1.21	23.39±1.07**	23.28±1.89
A+CUD	21.36±1.47**	21.73±1.32*	22.07±1.12**	22.39±1.39	22.87±1.42*	23.01±1.25	23.93±1.61**	24.03±1.71*	24.18±0.981**	25.81±1.42*	25.91±1.07*	26.36±1.03	26.48±0.902**
B+CUD	22.76±1.44*	22.91±1.51*	23.57±1.07	23.91±1.40**	23.96±1.31	24.03±1.61	24.18±1.42*	24.43±1.27	24.89±1.19	24.97±1.36*	24.91±1.17	24.88±1.13	24.80±1.09
G+CUD	22.97±1.37	23.07±1.27	23.46±1.21*	23.71±1.41**	23.96±1.31*	24.10±1.31*	24.57±1.19	24.81±1.17**	24.95±1.16*	24.89±1.27*	24.84±1.11	24.72±1.08*	24.67±0.941
H+CUD	22.81±1.21*	22.90±1.20*	22.96±1.17	23.07±1.31	23.48±1.32**	23.69±1.20	23.84±1.23	23.96±1.19	24.97±1.23	24.90±1.21	24.78±1.31**	24.67±1.08	24.60±1.01*

*Significant difference in comparison to control (*p≤0.5 and **p≤0.01)*

Table 2: Total erythrocyte count (TEC) (x 10^6/cumm) of experimental mice at an interval of 15 days (Mean±SE).

Groups/Days	0	15	30	45	60	75	90	105	120	135	150	165	180
Control	6.82±0.13	6.86±0.17	6.92±0.27	6.97±0.16	7.06±0.31	7.14±0.24	7.16±0.41	7.18±0.37	7.19±0.34	7.20±0.17	7.21±0.19	7.23±0.23	7.25±0.27
CUD (NO DEN)	6.29±0.52*	6.36±0.41*	6.52±0.63	6.70±0.13*	6.83±0.46	6.94±0.32	7.03±0.21**	7.19±0.61*	7.22±0.73*	7.25±0.49*	7.29±0.36	7.34±0.51**	7.40±0.42
DEN	6.87±0.51	6.89±0.62**	6.96±0.71*	6.98±0.18*	7.08±0.42*	7.11±0.52*	7.01±0.63	6.94±0.87*	6.66±0.33*	6.06±0.61	5.04±0.12**	4.71±0.43	3.61±0.46*
CUD	6.42±0.83	6.48±0.17	6.63±0.21*	6.69±0.47*	6.78±0.82*	6.83±0.27*	6.91±0.16*	7.03±0.51*	7.14±0.21	7.01±0.32**	6.81±0.27	6.71±0.61	6.68±0.53
A	6.31±0.37**	6.38±0.41	6.48±0.28*	6.63±0.46*	6.89±0.72*	6.96±0.69	7.09±0.33*	7.47±0.38	7.34±0.28	7.12±0.48	7.19±0.59*	7.23±0.72	7.32±0.58
B	6.33±0.61	6.45±0.42*	6.61±0.47	6.75±0.51	6.95±0.80	7.08±0.72**	7.22±0.61	7.45±0.56	7.29±0.51*	7.09±0.62	7.15±0.68*	7.21±0.52*	7.29±0.57**
G	6.39±0.41	6.41±0.39	6.54±0.41*	6.71±0.43	6.90±0.53	7.05±0.62**	7.17±0.47	7.39±0.54**	7.06±0.57	6.89±0.62	7.05±0.41*	7.15±0.46	7.20±0.37
H	6.37±0.52*	6.39±0.59*	6.53±0.47	6.68±0.45	6.70±0.52**	6.72±0.58	6.96±0.43	7.15±0.48	7.41±0.51**	7.69±0.48	7.35±0.61	7.23±0.42*	7.18±0.41**
A+CUD	6.31±0.97**	6.46±0.818	6.79±0.17*	6.98±0.42**	7.09±0.78	7.35±0.18*	7.49±0.19**	7.83±0.63*	7.98±0.76	8.38±0.84	8.73±0.39**	8.83±0.36*	8.90±0.47
B+CUD	6.37±0.53	6.40±0.61**	6.66±0.59	6.77±0.41*	6.81±0.47*	6.96±0.53	7.09±0.12	7.18±0.35	7.21±0.61*	7.40±0.69**	7.77±0.63	8.15±0.71	8.59±0.62*
G+CUD	6.42±0.59*	6.44±0.53	6.79±0.49	6.83±0.50	6.93±0.60	7.07±0.59*	7.18±0.57	7.31±0.51	7.77±0.53	7.86±0.59	7.72±0.47*	8.19±0.44	8.51±0.42
H+CUD	6.35±0.61	6.40±0.57	6.55±0.64**	6.72±0.51*	7.13±0.57	7.45±0.63	7.68±0.53*	7.88±0.56*	8.04±0.47**	8.13±0.52	8.29±0.56	8.44±0.48**	8.48±0.43*

*Significant difference in comparison to control (*p≤0.5 and **p≤0.01)*

Haematological parameters

Data of TEC is expressed in number of cellsx10^6/cumm and is mentioned in Table 2. Initially the TEC of control was 6.82±0.13 x 10^6/cumm and after 6 month, TEC of experimental mice was 7.25±0.27 x 10^6/cumm. In DEN treated (negative control) group the initial TEC at zero day of experiments was 6.87±0.51 x 10^6/cumm which decreased to 3.61±0.46 x 10^6/cumm significantly at the end of experiment. But in CUD treated group, the TEC at zero day was 6.29±0.52 x 10^6/cumm and 7.40±0.42 x 10^6/cumm at the end of experiment. CUD treated group in which the carcinogen had also been given, the initial TEC at zero day was 6.42±0.83 x 10^6/cumm. After the end of experiment, it was 6.68±0.53x 10^6/cumm. In test group A, the zero day

TEC was 6.31±0.37 x 10^6/cumm. The TEC decreased to 7.32±0.58 x 10^6/cumm at 180 day of experiment. In group CUD+A the total erythrocyte count, at zero day was 6.31±0.97 x 10^6/cumm which was increased to 8.90±0.47 x 10^6/cumm at the end of experiment. Group CUD+B had 6.37±0.53 x 10^6/cumm TEC at zero day and 8.59±0.62 x 10^6/cumm at the end of experiment, respectively. In CUD+G and CUD+H groups the TEC at zero day were 6.42±0.59 x 10^6/cumm and 6.35±0.61 x 10^6/cumm, respectively and were 8.51±0.42 x 10^6/cumm and 8.48±0.43 x 10^6/cumm at the end of the experiment. In group B, G and H, the TEC at zero day was found 6.33±0.61 x 10^6/cumm, 6.39±0.41 x 10^6/cumm and 6.37±0.52 x 10^6/cumm, respectively and at the end of experiment increased to 7.29±0.57 x 10^6/cumm, 7.20±0.37 x 10^6/cumm and 7.18±0.41 x 10^6/cumm.

Data of TLC is expressed in no. of cells x 10^3/cumm and is presented in Table-3.Initially the TLC count of control group was 8.91±0.30 x 10^3/cumm and after 6 month the TLC was 12.11±0.04 x 10^3/cumm. In DEN (negative control) group the initial TLC at zero day of experiments was 9.12±3.20 x 10^3/cumm which was decreased to 3.02±1.45 x 10^3/cumm. But in CUD treated group, the TLC at zero day was 8.65±4.31 x 10^3/cumm and 9.53±4.67 x 10^3/cumm at the end of experiment. In CUD treated group, the initial TLC at zero day was 9.02±5.31 x 10^3/cumm which decreased to 7.81±3.11 x 10^3/cumm. In test group like A, the zero day TLC was 9.08±5.68 x 10^3/cumm which was decreased to 8.64±3.08 x 10^3/cumm.CUD+A had the zero day mean TLC count as 8.97±4.02 x 10^3/cumm which was increased to 12.61±2.17 x 10^3/cumm, at the end of experiment. Group CUD+B has 8.89±6.31 x 10^3/cumm at zero day and was 10.55±3.18 x 10^3/cumm at the end of experiment.In group B, G and H, the TLC at zero day was observed as 9.01±6.81 x 10^3/cumm, 9.10±6.07 x 10^3/cumm and 9.07±7.03 x 10^3/cumm, respectively and at the end of experiment 8.59±3.19 x 10^3/cumm, 8.33±3.61 x 10^3/cumm and 8.30±4.1 x 10^3/cumm respectively. In groups CUD+G and CUD+H the TLC at zero day was 9.11±5.98 x 10^3/cumm, 9.03±6.19 x 10^3/cumm and was 10.21±3.81 x 10^3/cumm and 10.05±3.14 x 10^3/cumm at the end of the experiment, respectively.

Data of ALC is expressed in no. of cells x 10^3/cumm and is presented in Table-4.Initially the ALC count of control group was 4.30±0.69 x 10^3/cumm and after 6 month the ALC was 5.92±0.98 x 10^3/cumm. In DEN (negative control) group the initial ALC at zero day of experiments was 4.41±0.81 x 10^3/cumm which was decreased to 1.36±0.47 x 10^3/cumm. But in CUD treated group, the ALC at zero day was 4.13±0.70 x 10^3/cumm and 4.63±0.43 x 10^3/cumm at the end of experiment.In CUD treated group, the initial ALC at zero day was 4.43±0.84 x 10^3/cumm which decreased to 3.76±0.53 x 10^3/cumm. In test group like A, the zero day ALC was 4.42±0.91 x 10^3/cumm which was decreased to 4.19±0.27 x 10^3/cumm.CUD+A had the zero day mean ALC count as 4.39±0.87 x 10^3/cumm which was increased to 6.16±0.42 x 10^3/cumm, at the end of experiment. Group CUD+B has 4.36±0.89 x 10^3/cumm at zero day and was 5.16±0.80 x 10^3/cumm at the end of experiment.In group B, G and H, the ALC at zero day was observed as 4.40±0.94 x 10^3/cumm, 4.48±0.73 x 10^3/cumm and 4.44±0.84 x 10^3/cumm, respectively and at the end of experiment 4.18±0.94 x 10^3/cumm, 4.01±0.37 x 10^3/cumm and 3.98±0.77 x 10^3/cumm respectively. In groups CUD+G and CUD+H the ALC at zero day was 4.41±0.80 x 10^3/cumm, 4.42±0.79 x 10^3/cumm and was 4.90±0.28 x 10^3/cumm and 4.89±0.11 x 10^3/cumm at the end of the experiment, respectively.

Data of ANC is expressed in no. of cells x 10^3/cumm and is presented in Table-5.Initially the ANC count of control group was 4.57±0.72 x 10^3/cumm and after 6 month the ANC was 6.00±0.99 x 10^3/cumm. In DEN (negative control) group the initial ANC at zero day of experiments was 4.69±0.83 x 10^3/cumm which was decreased to 1.49±0.52 x 10^3/cumm. But in CUD treated group, the ANC at zero day was 4.38±0.78 x 10^3/cumm and 4.71±0.47 x 10^3/cumm at the end of experiment.

In CUD treated group, the initial ANC at zero day was 4.51±0.97 x 10^3/cumm which decreased to 3.88±0.56 x 10^3/cumm. In test group like A, the zero day ANC was 4.60±0.97 x 10^3/cumm which was decreased to 4.30±0.31 x 10^3/cumm.CUD+A had the zero day mean ANC count as 4.46±0.89 x 10^3/cumm which was increased to 6.25±0.45 x 10^3/cumm, at the end of experiment. Group CUD+B has 4.41±0.92 x 10^3/cumm at zero day and was 5.24±0.82 x 10^3/cumm at the end of experiment.In group B, G and H, the ANC at zero day was observed as 4.58±0.99 x 10^3/cumm, 4.56±0.75 x 10^3/cumm and 4.51±0.87 x 10^3/cumm, respectively and at the end of experiment 4.26±0.96 x 10^3/cumm, 4.11±0.38 x 10^3/cumm and 4.09±0.79 x 10^3/cumm respectively. In groups CUD+G and CUD+H the ANC at zero day was 4.52±0.84 x 10^3/cumm, 4.50±0.85 x 10^3/cumm and was 5.01±0.29 x 10^3/cumm and 4.96±0.12 x 10^3/cumm at the end of the experiment, respectively.

Table 3: Total leucocyte count (TLC) (x 10^3/cumm) count of experimental mice at an interval of 15 days (Mean±SE).

Groups/Days	0	15	30	45	60	75	90	105	120	135	150	165	180
Control	8.91±0.30	9.06±0.17	9.38±0.32	9.91±0.37	10.08±0.51	10.21±0.62	10.53±0.02	10.73±0.12	11.05±0.81	11.32±0.91	11.51±0.07	11.87±0.47	12.11±0.04
CUD (NO DEN)	8.65±4.31	8.71±4.36**	8.86±4.51*	8.93±4.70	9.08±5.01*	9.13±5.21	9.18±3.24*	9.20±4.22	9.27±4.56**	9.32±4.61	9.38±4.28	9.46±4.39*	9.53±4.67
DEN	9.12±3.20**	9.21±3.52*	9.43±4.07**	9.89±3.81	8.07±3.61	7.42±3.82	6.41±2.01	6.12±3.10*	5.87±2.12*	4.31±3.19	4.16±2.16	4.08±1.87*	3.02±1.45*
CUD	9.02±5.31*	9.13±5.27	9.31±5.17	9.67±4.81**	9.89±4.72	10.07±4.55	9.91±4.31**	9.73±4.24	9.41±4.27*	9.01±3.69	8.23±3.16**	8.11±3.08*	7.81±3.11
A	9.08±5.68*	9.15±5.51*	9.28±5.42	9.57±5.23	9.79±5.05**	9.98±4.93	9.78±4.61*	9.61±4.34	9.32±4.21*	8.91±4.07	8.83±3.21	8.79±3.12	8.64±3.08
B	9.01±6.81*	9.11±6.72	9.19±6.61**	9.41±6.52	9.61±6.37*	9.77±5.83*	9.58±5.61	8.91±5.37	8.87±5.21*	8.81±5.08	8.79±4.61*	8.68±4.52	8.59±3.19**
G	9.10±6.07	9.16±5.91*	9.24±5.82	9.33±5.74**	9.51±5.41*	9.67±5.34*	9.48±5.28	8.87±5.05	8.76±4.81	8.69±4.23*	8.59±4.07	8.41±3.82	8.33±3.61
H	9.07±7.03	9.11±6.87	9.15±6.67**	9.21±6.41	9.38±5.91	9.47±5.80	9.31±5.47*	8.83±5.32**	8.73±5.16	8.62±4.77*	8.57±4.41*	8.46±4.21*	8.30±4.10
A+CUD	8.97±4.02**	9.21±4.47	9.49±3.32	9.89±3.77*	10.08±2.01*	10.33±2.61*	10.69±3.16*	10.93±4.03	11.31±2.12**	11.67±3.41*	11.91±4.91*	12.25±3.53*	12.61±2.17**
B+CUD	8.89±6.31*	9.12±6.27*	9.25±6.08	9.49±5.61*	9.68±5.32*	9.87±5.17*	9.67±5.03	9.47±4.71	9.73±4.57	9.84±4.31	10.08±4.17	10.67±4.08*	10.55±3.18
G+CUD	9.11±5.98	9.18±5.81*	9.27±5.61	9.39±5.47*	9.59±5.27	9.71±5.17	9.51±5.09**	9.30±4.81	9.26±4.67	9.42±4.51	10.02±4.41**	10.17±4.32*	10.21±3.81*
H+CUD	9.03±6.19	9.13±6.01	9.19±5.81**	9.27±5.72	9.44±5.51	9.59±5.42*	9.40±5.31	9.31±5.06*	9.12±4.87*	9.09±4.62*	9.51±4.41	9.88±4.17	10.05±3.14

*Significant difference in comparison to control(*p≤0.5 and **p≤0.01)*

Table 4: Absolute lymphocyte count (ALC) (x 10^6/cumm) of experimental mice at an interval of 15 days (Mean±SE).

Groups/Days	0	15	30	45	60	75	90	105	120	135	150	165	180
Control	4.30±0.69	4.41±0.42	4.59±0.24	4.81±0.49	4.92±0.19	4.98±0.33	5.17±0.54	5.23±0.68	5.41±0.57	5.53±0.28	5.62±0.31	5.81±0.73	5.92±0.98
CUD (NO DEN)	4.13±0.70*	4.24±0.72	4.31±0.69*	4.37±0.58*	4.43±0.42	4.45±0.39*	4.48±0.69**	4.49±0.84	4.52±0.92*	4.54±0.14	4.55±0.28*	4.61±0.63*	4.63±0.43
DEN	4.41±0.81*	4.43±0.61	4.56±0.71	4.86±0.24*	3.92±0.92**	3.59±0.43*	3.05±0.64	2.94±0.59	2.81±0.36	2.08±0.31*	1.92±0.27*	1.90±0.17	1.36±0.47*
CUD	4.43±0.84*	4.41±0.64	4.54±0.84	4.74±0.18	4.83±0.29	4.85±0.36	4.85±0.47*	4.72±0.53*	4.59±0.61	4.35±0.74*	3.96±0.89	3.89±0.92	3.76±0.53
A	4.42±0.91	4.44±0.738	4.51±0.42**	4.67±0.75	4.78±0.94*	4.86±0.86	4.78±0.73	4.66±0.21**	4.53±0.28*	4.36±0.69	4.28±0.74**	4.25±0.18**	4.19±0.27*
B	4.40±0.94	4.42±0.57	4.44±0.55	4.60±0.85	4.69±0.69	4.77±0.54**	4.65±0.22*	4.33±0.51	4.34±0.49*	4.26±0.16	4.24±0.21*	4.22±0.86	4.18±0.94*
G	4.48±0.73*	4.41±0.82**	4.51±0.98*	4.56±0.37	4.64±0.76	4.76±0.41	4.62±0.47*	4.32±0.53	4.27±0.84	4.23±0.61	4.15±0.65	4.10±0.21*	4.01±0.37*
H	4.44±0.84	4.40±0.34*	4.48±0.76	4.50±0.26**	4.54±0.86*	4.63±0.16	4.56±0.36	4.30±0.61*	4.24±0.42*	4.15±0.69**	4.16±0.48	4.09±0.59*	3.98±0.77
A+CUD	4.39±0.87	4.49±0.29	4.62±0.59	4.84±0.43*	4.90±0.33	5.02±0.77	5.21±0.61	5.33±0.98	4.55±0.71	5.71±0.14	5.83±0.21*	5.98±0.63	6.16±0.42**
B+CUD	4.36±0.89**	4.47±0.19*	4.51±0.41**	4.62±0.82*	4.74±0.27	4.83±0.87*	4.72±0.91**	4.61±0.84**	4.73±0.16**	4.76±0.72	4.91±0.18	5.21±0.68*	5.16±0.80
G+CUD	4.41±0.80*	4.48±0.47	4.53±0.63	4.58±0.92	4.65±0.46**	4.74±0.18*	4.66±0.78	4.56±0.20	4.52±0.91	4.57±0.39*	4.87±0.65*	4.91±0.21	4.90±0.28
H+CUD	4.42±0.79	4.45±0.77	4.48±0.27*	4.52±0.39	4.61±0.65	4.69±0.23	4.59±0.88	4.54±0.49	4.46±0.64*	4.43±0.72*	4.63±0.47	4.86±0.30*	4.89±0.11*

*Significant difference in comparison to control (*p≤0.5 and **p≤0.01)*

Table 5: Absolute Neutrophil count (ANC) (x 10^6/cumm) of experimental mice at an interval of 15 days (Mean±SE).

Groups/Days	0	15	30	45	60	75	90	105	120	135	150	165	180
Control	4.57±0.72	4.50±0.43	4.67±0.29	4.90±0.51	5.00±0.23	5.07±0.38	5.22±0.55	5.32±0.69	5.50±0.59	5.61±0.37	5.71±0.37	5.90±0.77	6.00±0.99
CUD (NO DEN)	4.38±0.78**	4.33±0.74**	4.40±0.72	4.44±0.60	4.52±0.45*	4.53±0.43	4.56±0.72	4.57±0.86*	4.60±0.97	4.62±0.19	4.67±0.34	4.70±0.65	4.71±0.47*
DEN	4.69±0.83	4.56±0.65**	4.68±0.75	4.92±0.26	4.01±0.96	3.68±0.46	3.14±0.67**	3.00±0.61*	2.90±0.41	2.13±0.34	2.00±0.31	1.99±0.23*	1.49±0.52
CUD	4.51±0.87	4.50±0.69*	4.60±0.89*	4.81±0.21	4.92±0.31	4.99±0.37	4.93±0.49	4.83±0.57	4.67±0.66**	4.47±0.77	4.07±0.92**	3.99±0.95*	3.88±0.56**
A	4.60±0.97*	4.53±0.79	4.61±0.46	4.75±0.77	4.87±0.98	4.96±0.89*	4.86±0.74**	4.78±0.26	4.61±0.32	4.42±0.75*	4.39±0.77	4.36±0.23	4.30±0.31
B	4.58±0.99*	4.51±0.58**	4.56±0.57**	4.68±0.87*	4.78±0.69*	4.85±0.56	4.77±0.26	4.40±0.55	4.40±0.51	4.38±0.19*	4.35±0.26	4.31±0.89	4.26±0.96
G	4.56±0.75	4.54±0.86	4.62±0.99	4.64±0.39*	4.73±0.79*	4.80±0.43**	4.71±0.49	4.41±0.54**	4.35±0.85*	4.31±0.66*	4.26±0.66	4.18±0.24	4.11±0.38
H	4.51±0.87**	4.52±0.37	4.53±0.80*	4.58±0.29	4.66±0.88	4.71±0.17*	4.61±0.38	4.39±0.63	4.32±0.46	4.27±0.74	4.25±0.53*	4.20±0.61	4.09±0.79
A+CUD	4.46±0.89*	4.58±0.31*	4.70±0.61**	4.92±0.45*	4.99±0.35**	5.11±0.78*	5.32±0.64*	5.41±0.99*	5.62±0.74*	5.80±0.15**	5.91±0.23	6.10±0.65**	6.25±0.45*
B+CUD	4.41±0.92	4.51±0.23	4.63±0.46	4.73±0.86	4.80±0.29*	4.91±0.89	4.80±0.93	4.70±0.86	4.81±0.18	4.89±0.74*	5.00±0.20*	5.30±0.69	5.24±0.82

G+CUD	4.52±0.84	4.56±0.49	4.61±0.66*	4.67±0.95*	4.77±0.48	4.80±0.20	4.73±0.79**	4.62±0.23	4.60±0.95*	4.68±0.44	4.98±0.67	5.00±0.24*	5.01±0.29*
H+CUD	4.50±0.85*	4.54±0.81*	4.56±0.29	4.61±0.40	4.70±0.66*	4.75±0.25	4.67±0.90	4.61±0.50*	4.53±0.65	4.51±0.73	4.72±0.49**	4.95±0.32	4.96±0.12

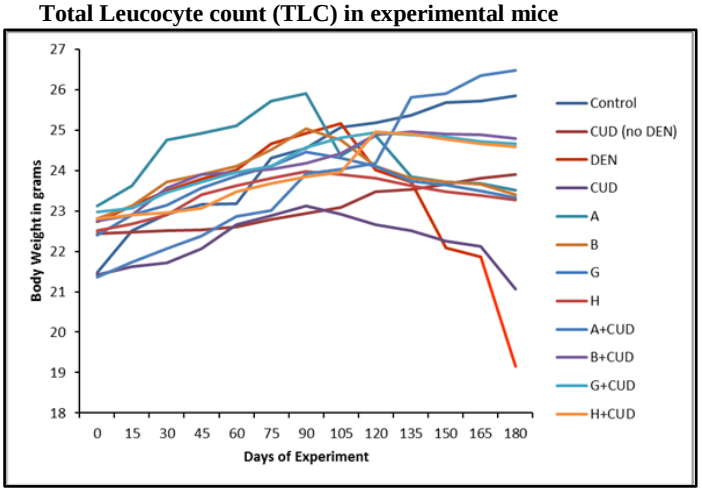
Table 6: Haemoglobin content (Hb) of experimental mice at regular interval of 15 days (gm%, mean±SE).

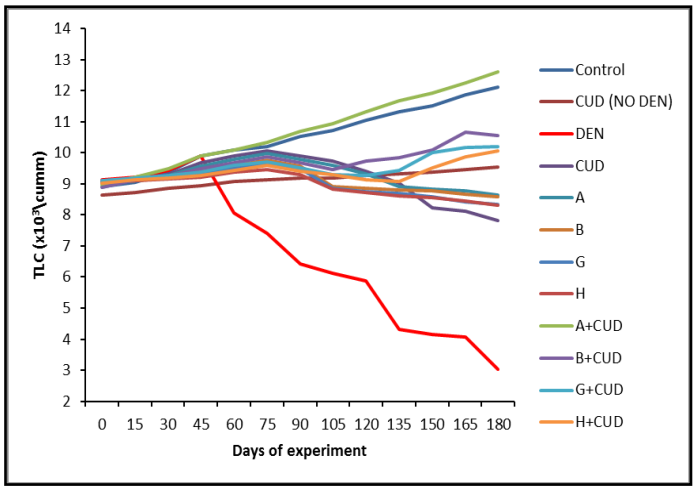
Groups/Days	0	15	30	45	60	75	90	105	120	135	150	165	180
Control	12.45±1.42	12.47±1.51	12.53±1.52	12.51±1.42	12.55±1.47	12.50±1.44	12.52±1.41	12.55±1.37	12.56±1.35	12.58±1.39	12.62±1.34	12.66±1.21	12.68±1.36
CUD(NO DEN)	12.19±1.31*	12.21±1.42	12.27±1.51*	12.49±1.36	12.62±1.47*	12.85±1.51	12.99±1.62*	13.41±1.73*	13.53±1.81*	13.66±1.74*	13.70±1.32	13.76±1.21*	13.78±1.19
DEN	12.40±1.36*	12.42±1.38	12.43±1.29	12.45±1.31**	12.46±1.33	12.25±1.36*	12.08±1.39	11.82±1.36*	11.65±1.30	10.52±1.32*	10.09±1.21	9.36±1.19*	8.02±1.07
CUD	12.32±1.31	12.45±1.28**	12.86±1.33	12.90±1.37	13.06±1.29*	13.40±1.42	13.87±1.47	13.67±1.40*	13.46±1.33**	13.22±1.28*	12.79±1.21*	12.34±1.28	12.09±1.19**
A	12.27±1.17**	12.43±1.27	12.84±1.29	12.87±1.32*	13.07±1.33	13.38±1.38*	13.85±1.41	14.04±1.43	13.95±1.47	13.80±1.27	13.76±1.23*	13.71±1.19	13.63±1.13
B	12.31±1.40	12.39±1.35*	12.79±1.25	12.83±1.38**	13.03±1.27	13.30±1.30	13.79±1.37**	14.02±1.39*	13.95±1.41*	13.75±1.26	13.71±1.19**	13.67±1.21**	13.56±1.18
G	12.34±1.28*	12.37±1.21	12.72±1.24**	12.79±1.41	13.04±1.32**	13.25±1.19**	13.72±1.29	13.96±1.26	13.91±1.25*	13.70±1.32*	13.65±1.27	13.61±1.21	13.49±1.29
H	12.34±1.42	12.36±1.40	12.68±1.23**	12.74±1.21	12.95±1.31*	13.10±1.22	13.67±1.28	13.87±1.27	13.82±1.30	13.65±1.33*	13.59±1.24**	13.55±1.32*	13.40±1.25
A+CUD	12.41±1.48*	12.48±1.47	12.89±1.36**	12.94±1.39	13.08±1.33*	13.42±1.41*	13.89±1.52*	14.06±1.47**	14.64±1.37	14.82±1.42	15.07±1.37	15.21±1.27	15.38±1.31*
B+CUD	12.29±1.42*	12.40±1.37	12.81±1.27	12.85±1.36**	13.05±1.31	13.35±1.32	13.81±1.39	14.01±1.41	14.22±1.42	14.48±1.25	14.74±1.21*	14.89±1.22*	14.90±1.17*
G+CUD	12.27±1.41	12.36±1.39**	12.76±1.21	12.80±1.29*	13.01±1.25	13.27±1.28	13.76±1.31*	13.98±1.30*	14.03±1.27*	14.33±1.21**	14.69±1.23	14.74±1.17	14.86±1.14
H+CUD	12.30±1.39*	12.35±1.35	12.70±1.27*	12.76±1.23	12.98±1.33**	13.12±1.29*	13.70±1.20	13.90±1.24	13.97±1.26	14.08±1.31	14.62±1.28*	14.70±1.22	14.78±1.11**

*Significant difference in comparison to control (*p≤0.5 and **p≤0.01)*

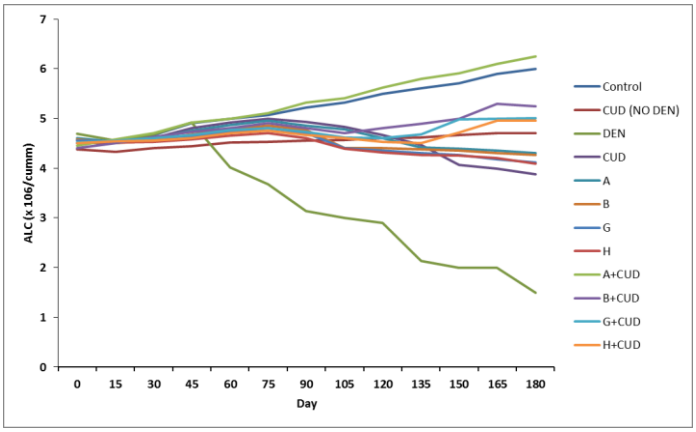
Data of Hemoglobin is expressed in gm% and is presented in Table-6. Initially the Hb content of control was 12.45±1.42gm% and after 6 month the Hb content of mice was 12.68±1.36gm%. In DEN (negative control) group the initial Hb content at zero day of experiment was 12.40±1.36gm%, which decreased to 8.02±1.07gm%, at the end of experiment. But in CUD treated group, their Hb content at zero day was 12.19±1.31gm% and 13.78±1.19gm% at the end of experiment. In CUD treated group in which the carcinogen has been given, the initial Hb content at zero day was 12.32±1.31gm%, after the end of experiment the Hb content was 12.09±1.19gm%. In test group A, the zero day Hb content was 12.27±1.17gm%. The Hb content increased to 13.63±1.13gm% at the end of experiment. In group B, G and H, the Hb content at zero day was found 12.31±1.40gm%, 12.34±1.28gm% and 12.34±1.42gm%, respectively and at the end of experiment the Hb content reached to 13.56±1.18gm%, 13.49±1.29gm% and 13.40±1.25gm%, respectivelyCUD+A had Hb content at zero day 12.41±1.48gm% which was increased to 15.38±1.31gm%, at the end of experiment. Group CUD+B had 12.29±1.42gm% Hb content on zero day which was increased to 14.90±1.17gm% at the end of experiment. In CUD+G and CUD+H, the Hb content at zero day was 12.27±1.41gm%, 12.30±1.39gm% and was 14.86±1.14gm% and 14.78±1.11gm% at the end of the experiment, respectively.

Total Leucocyte count (TLC) in experimental mice

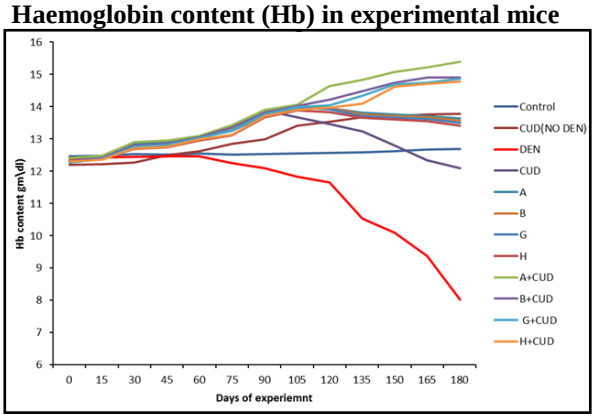




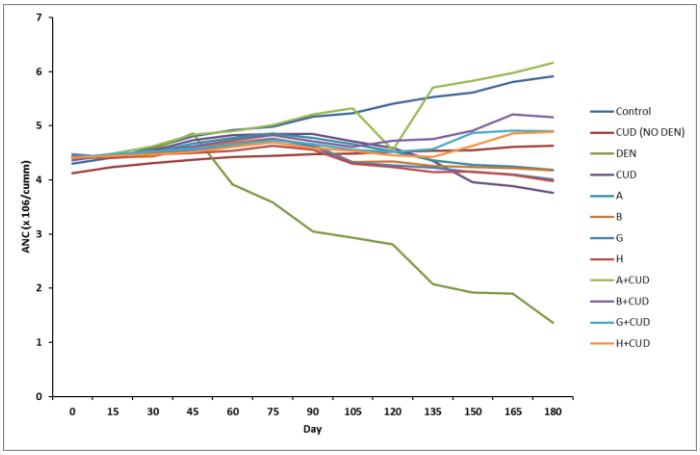
Absolute lymphocyte count (AlC) of experiment of mice.



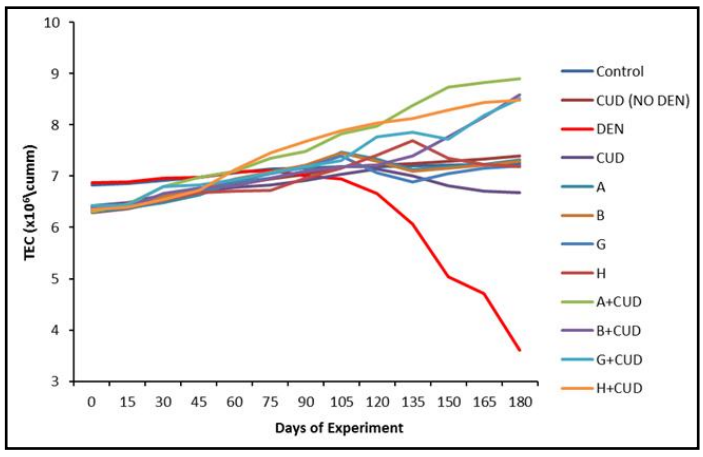
Haemoglobin content (Hb) in experimental mice



Absolute neutrophil count (AlC) of experiment of mice.



Total erythrocyte count (TEC) in experimental mice



Discussion

In-vivo study with *T. baccata* leaves and bark extracts alone and in combination with CUD were carried out in experimental mice for a period of six months. With an observation at 15 days interval in mice, an attempt was made to produce cancer using DEN and various clinicohematological parameters were observed. The body weight of mice was decreased substantially in DEN treated mice indicating in the development of cancer, due to DEN.Ramji and You, (1992) reported that aflatoxin has been directly related to under weight status in children in Benin and Togo. Bedi et al.,(1996) reported decreased in body weight in Guinea fowl fed on aflatoxin B1. In present study body weight in DEN treated mice was decreased at the end of experiment. However, there was increase in body weight in other test groups. This study showed that the weight loss in DEN treated group may be due to the carcinogenic effect of DEN; however, herbal formulations of extracts and CUD were found to be a preventive agent against the carcinogenic effects of DEN. DEN is already known chemical carcinogen.Increased immunocompetence of an individual is a very essential parameter to prevent the development of cancers by several mechanisms, of which the upregulation of lymphocyte proliferation and stimulation activity, increased macrophage activity, higher antibody production and increased synthesis and secretion of cytokines (IL-1, Il-2) plays significant role by enhancing the recognition of tumor cells by the immune cells of the body and cytotoxic activities of the tumor killing cells, the lymphocytes. Using herbs for cancer treatment can help the body to support its

healing power. In the present investigation, both doses of QC (5 and 25 mg/kg) led to a significant decrease in the number as well as the mean area of GST-P positive foci, TUNEL positive apoptotic cells, p53 positive hepatocytes, and restoration of cellular morphology. These results clearly indicate that quercetin inhibits diethylnitrosamine-induced hepatic preneoplastic lesions in medium-term rat liver bioassay. In the mice given *T. baccata*alone and along with CUD. The body weight either remain constant or enhanced substantially. These preparations as shown in the *in-vitro* study were having anti-carcinogenic effect, which might be altering the clinoco effects of the cancer caused by DEN.

Various hematological parameters indicated the leukocytosis, erythrocytosis higher hemoglobin content in treated mice with *T. baccata* products along with indigenous cow urine. While, in DEN treated mice there was leucopenia, erythropenia and decreased heamoglobin content. These findings are further supported by the fact that CUD had the immunomodulatory property which caused leukocytosis leading to the control of the highly proliferating cells through their destruction by the white blood cells. Erythrocytosis and increased hemoglobin content are the indication of good health and recovery and neutralization of the effect of DEN by *T. baccata* and CUD. Joshi *et al*., 2013 investigated that the immunomodulatory effect of distilled Gir cow urine in rabbits throughhaematological parameters. The study revealed that the values of total leucocyte count (TLC), absolute lymphocyte count (ALC) and absolute neutrophil count (ANC) were significantly increased in Group II, in which the rabbits were given Gir cow urine distillate alone and Gir cow urine distillate with citric acid, respectively. In the present study the ALC and ANC increased 40.31% and 40.13% inn extracts and/or CUD treated mice in comparison to control or DEN treated mice. Increase in TEC and Hb content is an indication of enhanced vitality of mice. Similarly leukocytosis, lymphocytosis and neutrophilia are the immune cell showing immunopoturtration, which is considered protective against cancer and an indication of a good prognosis (chauhan, 2005). It further needs a detailed study for further confirmation.

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