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IN VIVO EVALUATION OF CO (II) COMPLEXES OF B-DIKETONATE DERIVATIVES AS POTENTIAL ANTITUMOR AGENT AGAINST BENZO[A]PYRENE INDUCED EXPERIMENTAL LUNG CARCINOGENESIS

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ABSTRACT
The in vivo antitumor efficacy of the Co(II) β-diketonate complex $Co(dpq)_2(acac)_2$ [C2] was evaluated against benzo[a]pyrene-induced lung carcinogenesis in female Swiss albino mice. Antitumor activity was assessed by histopathlogical, hematological parameters, serum biochemical markers and survival. C2 treatment significantly reduced tumor burden, enhanced tumor growth inhibition, and prolonged survival compared with the disease control group. Its therapeutic effects were comparable to or better than cisplatin, demonstrating promising antineoplastic activity with a favorable therapeutic profile.

KEYWORD: Co (II) metal complexes, Benzopyrene, lung tumor, Mean Survival Time.

INTRODUCTION

Cancer remains a major global health challenge due to its uncontrolled proliferation, transformation, and metastatic potential. According to IARC, approximately 20 million new cancer cases and 9.7 million cancer-related deaths were reported in 2022, with lung cancer being the most commonly diagnosed cancer (2.5 million cases) worldwide[1]. Although surgery remains the primary treatment for localized tumors, chemotherapy continues to be essential for advanced disease and preventing recurrence. Benzo[a]pyrene (B[a]P), a potent polycyclic aromatic hydrocarbon carcinogen, promotes lung carcinogenesis through oxidative stress-mediated damage[2]. Metal-based chemotherapeutics, particularly bioessential transition metal complexes, have emerged as promising alternatives due to their biological activity and reduced toxicity [3-8]. In our previous study, the Co(II) β-diketonate complex $Co(dpq)_2(acac)_2$ (C2) demonstrated potent anticancer activity in vitro with minimal toxicity [9]. Based on its selective activity against lung cancer cells, the present study further evaluates it's in vivo antitumor efficacy against B[a]P-induced lung carcinogenesis in Swiss albino mice.

2. MATERIALS AND METHODS

2.1 Chemicals

Benzo[a]pyrene (~96% purity) and cisplatin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Corn oil and other chemicals were of analytical grade obtained from commercial sources. Hematological and biochemical analyses were performed using automated diagnostic instruments at the Veterinary Clinical Complex and Department of Veterinary Pathology, Assam Agricultural University, Guwahati.

2.2 Animals:

Healthy female Swiss albino mice (6–8 weeks old) were obtained from the Animal House, Department of Zoology, Gauhati University, Assam, India. Animals were maintained under controlled temperature, humidity, and 12 h light/dark cycle conditions with free access to standard diet and water. Mice were acclimatized for 7 days before experimentation. All procedures were performed following Institutional Animal Ethical Committee approval (IAEC/2024/Ethical-Per/PhD-IAEC/2024-1).

2.3 Determination of median lethal doses (LD50)

The acute toxicity and LD_{50} of C2 were determined following a previously described method [10]. The compound was administered intraperitoneally in 3% DMSO at escalating doses, and animals were monitored for behavioral changes, toxicity signs, and mortality. The LD_{50} value was previously determined as 121.67 mg/kg. Based on acute and subacute toxicity studies, low (6.08 mg/kg; 1/20th LD_{50}) and high (12.17 mg/kg; 1/10th LD_{50}) doses were selected for in vivo anticancer evaluation. The antitumor efficacy of C2 against Benzo[a]pyrene-induced lung cancer was assessed through tumor growth inhibition, survival analysis, histopathological examination, hematological parameters, and serum biochemical profiling.

2.4 Experimental design:
Experimental animals were divided into five groups (n=12/group), with one control group and four benzo[a]pyrene-induced tumor groups. C2 treatment was administered intraperitoneally after 13th weeks of tumor induction. Body weight was monitored weekly throughout the study. At the end of the experiment after 16th week, six mice from each group were sacrificed, and blood samples were collected for hematological and biochemical analyses. Lungs were excised, weighed, homogenized in phosphate buffer, and centrifuged to obtain tissue extracts for biochemical evaluation.

- Group 1 (normal control): animals received DMSO intraperitoneally from 13th week of the experiment till 16th week.
- Group 2 (carcinogen control): animals were administered with B(a)P (50 mg/kg body weight dissolved in corn oil) orally twice a week for four successive weeks for tumor induction[11].
- Group 3 (positive control): animals were administered with B(a)P (as in Group 2) along with Cisplatin (6 mg/kg body weight, intraperitoneally) once in a week from 13th week after B(a)P administration till the end of the study period.
- Group 4 (compound C2 low dose): animals were treated with B(a)P (as in Group 2) along with compound C2 (6.08 mg/kg body weight, intraperitoneally) but from 13th week of Benzopyrene induction on alternative days and continued till the end of experiment i.e., 16th week.
- Group 5 (compound C2 high dose): animals were treated with B(a)P (as in Group 2) along with compound C2 (12.17 mg/kg body weight, intraperitoneally) from 13th week of Benzopyrene induction on alternative days for 16 week.

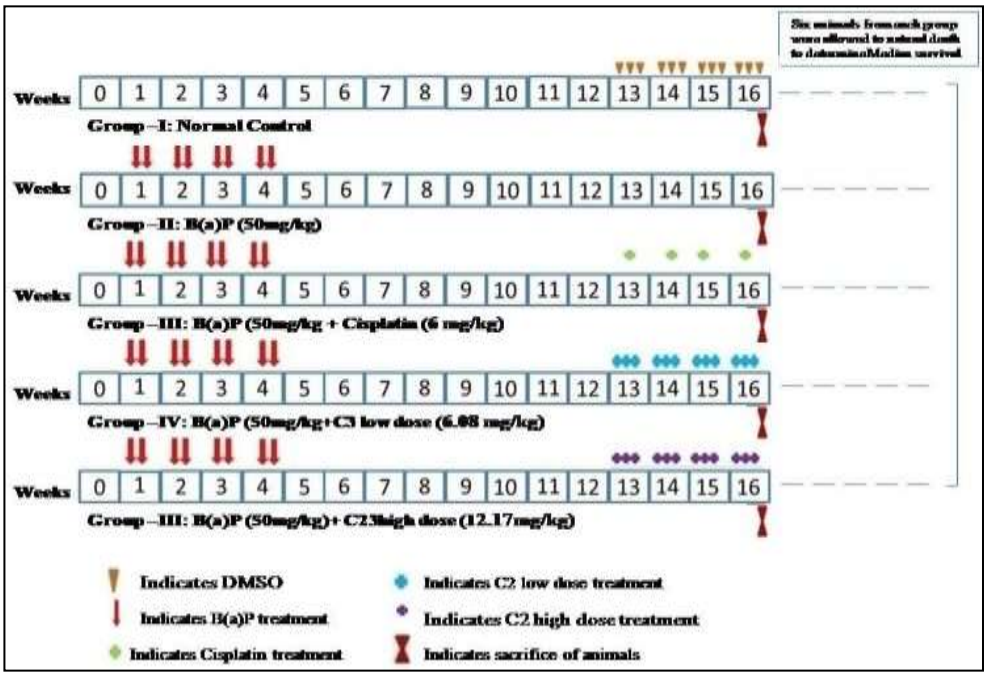


Figure1: Experimental design of the study

Rest of the animals was allowed to natural death for determination of mean survival time (MST) and percentage inc rease life span (%ILS) [12]

2.5 Body weight
Body weight of each mouse was recorded at the beginning of the experiment and monitored weekly until sacrifice. The average change in body weight was calculated for each group.

2.6 Lung weight
Animals were euthanized at the end of the experiment. Entire lungs were excised by opening the thoracic cavity carefully and washed gently with cold PBS or normal saline to remove blood. Lungs were blot dried using filter paper. The whole lung weight was measured using an analytical balance. Lung weights of animals from control and experimental groups were measured and compared.

2.7 Tumor incidence
Tumor incidence was recorded and compared among all experimental groups.

2.8 Mean survival time (MST) and Percentage increase in life span (%ILS)
Mean survival time (MST) and percentage increase in life span (% ILS) were calculated to evaluate the antitumor efficacy of C2 compared with the B(a)P control group using the following formulas[13]
MST = (Day of first death + Day of last death)/2
% ILS = [(MST of treated group / MST of control group) − 1] × 100

2.9 Median survival study

The remaining six mice from each group were monitored for survival up to 40 weeks following B[a]P administration. Animals showing severe tumor progression and no response were euthanized and reco
rded as deaths. Survival data were analyzed using the Kaplan–Meier method in GraphPad Prism, and median survival time was calculated [14]

3. STATISTICAL ANALYSIS

In vivo data (n = 6/group) were expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test in GraphPad Prism 9. A p-value < 0.05 was considered statistically significant.

4. RESULTS

The antitumor efficacy of cobalt complex C2 was evaluated in a Benzo[a]pyrene B(a)P-induced lung cancer mouse model by assessing lung weight, body weight, histopathology, hematological, and biochemical parameters. The lung tumor model was established through oral administration of B(a)P for four weeks. Compared with normal lungs, B[a]P-treated mice showed visible tumor development (Figure 2), increased tumor burden, and marked histopathological alterations, including alveolar distortion and hyper chromatic nuclei, confirming successful tumor induction (Figure 3).

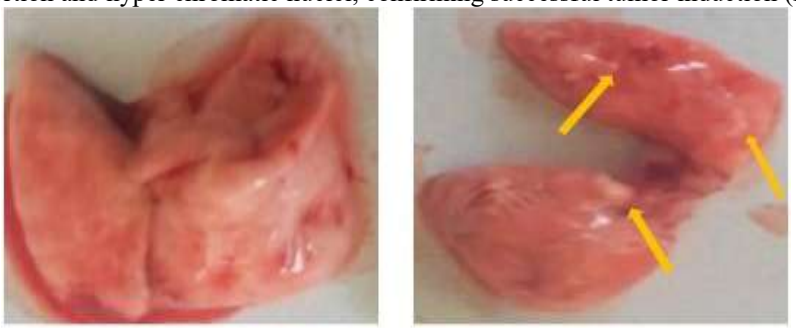
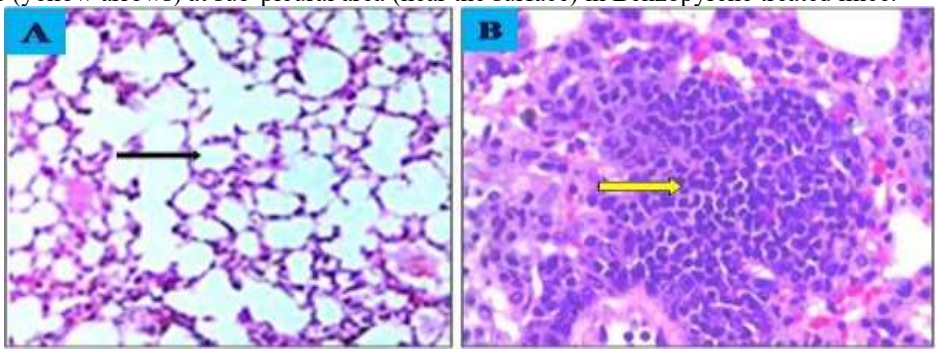


Figure 2: Mature lungs of female Swiss Albino mice (A) Normal lungs of mice without any treatment and (B) Lungs with mature tumor (yellow arrows) at sub-pleural area (near the surface) in Benzopyrene treated mice.



(A)Normal (B) Benzopyrene treated Figure

Figure 3: Histopathology (TS) of (A) normal Lung tissue and (B) Benzopyrene treated Adeno carcinoma cell. (A) Normal lung alveoli with interstitial tissue (black arrow), while (B) Alveolar damage was seen with increased number of hyper chromatic nuclei (yellow arrow) in the cells of alveolar wall.

4.1 General observations:

Body weight, lung weight, and tumor incidence were evaluated to assess tumor progression in B(a)P-induced lung cancer-bearing mice. B(a)P administration resulted in a significant ($p<0.001$) decrease in body weight with increased lung weight and tumor incidence compared with controls. Treatment with C2 at both low and high doses significantly reduced tumor-associated changes, with high-dose C2 showing better efficacy than low-dose C2 and comparable or superior effects to cisplatin.

Table 1: Effect of the low and high doses of tested compound C2 on body weight and lung weight in control and experimental group of animals.

Parameters	Normal control	B(a)P control	Cisplatin treated	C2 (low dose)	C2 (high dose)
Body weights(g)	29.09 ± 1.04	$20.50\pm1.43^{a,d}$	$25.82\pm1.09^{b,d}$	$24.63\pm1.36^{a,d,e}$	$26.78\pm1.37^{c,d,e}$
Lung weights(mg)	259.67±6.19	$351.67\pm4.27^{a,c}$	$275.2\pm5.91^{c,d}$	$277\pm2.36^{a,c,e}$	$267.7\pm2.87^{b,c,d,e}$
Number of animals	6	6	6	6	6

Tumor incidence	0	100	50	33	16.66

All the values are expressed as a mean S.D. for six mice in each group. Statistical significance was determined by one-way ANOVA followed by Tukey post hoc test.

4.1.1 Body weight

B(a)P-induced tumor-bearing mice showed a significant reduction in body weight ($p<0.0001$) compared with control animals, reaching 20.50g at the end of the study (table1). Cisplatin treatment partially restored body weight (25.82 g), whereas C2 treatment showed greater recovery, with low and high-dose groups reaching 24.63g and 26.78g, respectively (Figure 4). These findings indicate that C2 effectively restored body weight in B(a)P-induced mice in a dose-dependent manner and C2 high dose showed better result than cisplatin.

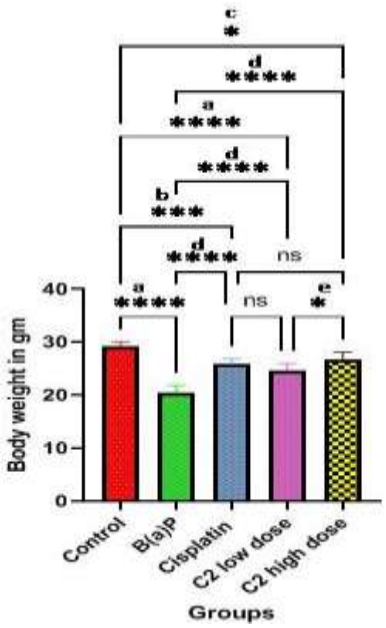


Figure 4: Results of study of different groups on body weights in female Swiss Albino Mice. All the values are expressed as a mean S.D. for six mice in each group. Statistical significance was determined by one-way ANOVA followed by Tukey post hoc test; where: [a]$p<0.0001$ when compared with normal control group, [b]$p<0.001$ when compared with normal control group, [c]$p<0.05$ normal control group, [d]$p<0.0001$ when compared with B(a)P control group, [e]$p<0.05$ when compared low and high doses groups of complex C2.

4.1.2 Lung weights

B(a)P administration significantly increased lung weight (351.67 mg; $p<0.0001$) compared with vehicle control mice (259.67 mg), indicating tumor progression (table1). Cisplatin reduced lung weight to 275.2 mg, whereas C2 treatment showed greater reduction, with low- and high-dose groups showing 277 mg and 267.7 mg, respectively (Figure 5). Both doses of C2 significantly decreased lung weight. But compared to cisplatin, high dose exhibiting superior efficacy while Cisplatin showed better results than C2 low dose.

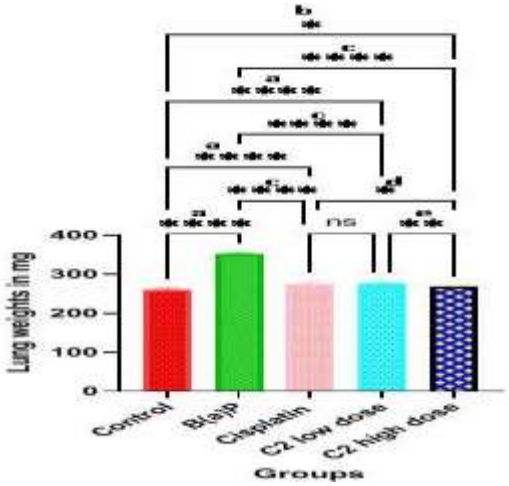


Figure 5: Results of study of different groups on lung weights in female Swiss Albino Mice. All the values are expressed as a mean S.D. for six mice in each group. Statistical significance was determined by one-way ANOVA followed by Tukey post hoc test. [a]$p<0.0001$ when compared with normal control group, [c] c normal control group, [c]$p<0.0001$ when

compared with B(a)P control group, ns= no significance when compared drug control group cisplatin with C2 low dose, drug control group cisplatin C2 low dose, [d]p<0.05 when compared with drug control group cisplatin with C2 high dose and [e]p<0.01 when compared between low and high doses groups of complex C2.

4.2 Antitumor activity

The antitumor efficacy of cobalt complex C2 was evaluated in a B(a)P-induced lung cancer mouse model by assessing tumor growth inhibition, histopathological changes, hematological and biochemical parameters, and survival rate. The tumor model was established by oral administration of B(a)P for one per four weeks (Figure 2 and 3). Treatment with C2 at both low and high doses significantly reduced tumor incidence compared with the B(a)P group, with a dose-dependent improvement in antitumor activity, demonstrating the tumor growth inhibitory potential of C2 (Figure 6).

4.2.1 Histopathological evaluation

Histopathological analysis of lung tissues by H&E staining [15] (Figure 6) revealed normal architecture with uniform nuclei in control mice. B(a)P-induced tumor-bearing mice showed disrupted alveolar structure and increased hyperchromatic nuclei, indicating tumor progression (Figure 6C and 6D). Treatment with low and high doses of C2 significantly reduced alveolar damage and restored near-normal lung architecture, with high-dose C2 showing better tumor inhibitory effects (Figure 6E).

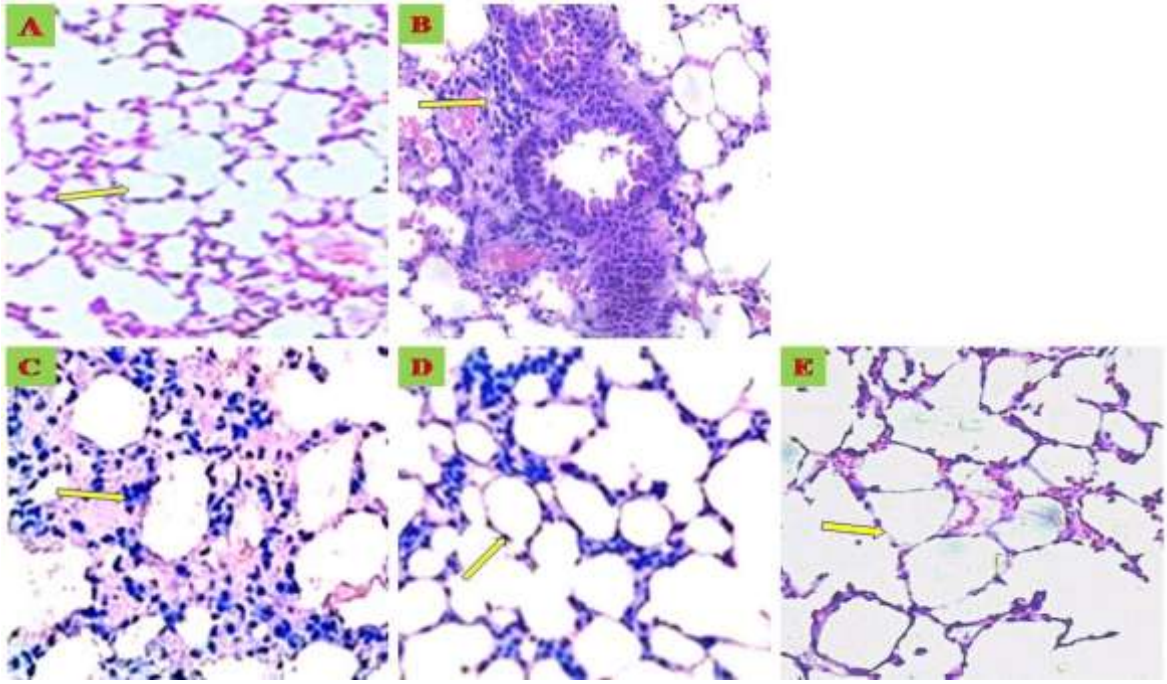


Figure 6: Effect of compounds on the histology of lung sections at 10X from control and experimental groups of mice. Normal control mice lung sections showing regular architecture of alveoli with uniform nuclei and interstitial tissue (arrow) (Figure A). B(a)P-induced lung cancer bearing animals showed loss of architecture and alveolar damage as seen from increased number of hyper chromatic nuclei in the cells of alveolar wall (Figure B). Cisplatin treated animals showing better architecture of alveolus and interstitial tissue than B(a)P treated with reduced number of hyper chromatic nuclei (Figure C). Low dose of Compound C2 treated animals exhibited reduced alveolar damage with decreased number of hyper chromatic nuclei and thickening of interstitial tissue similar to that of normal control animals (Figure D). High dose of Compound C2 treated animals exhibited near normal architecture with much reduced alveolar damage and reduced number of hyper chromatic nuclei and restored interstitial tissue (Figure E).

4.3 Results of Survival Study

Survival analysis was performed for 40 weeks following B(a)P administration to evaluate the long-term antitumor efficacy of C2. Treatment with C2 and cisplatin significantly increased the mean survival time of tumor-bearing mice compared with the B(a)P control group. C2-treated groups also showed a significant percentage increase in lifespan (ILS %), confirming its anticancer potential. No significant difference was observed between the two C2 dose groups, indicating comparable survival benefits (Table 3).

Table 3. Effects of the test compound on the survival time of EAC-bearing mice.

GROUPS	Mean survival time (MST), day (Mean±SE)	Increase of life span (ILS),%
B(a)P treated	29.66±6.95	-
Cisplatin	41.77±9.33	40.75%

C2 low dose	46.22±8.16	56.06%
C2 high dose	46.67±7.75	61.84%

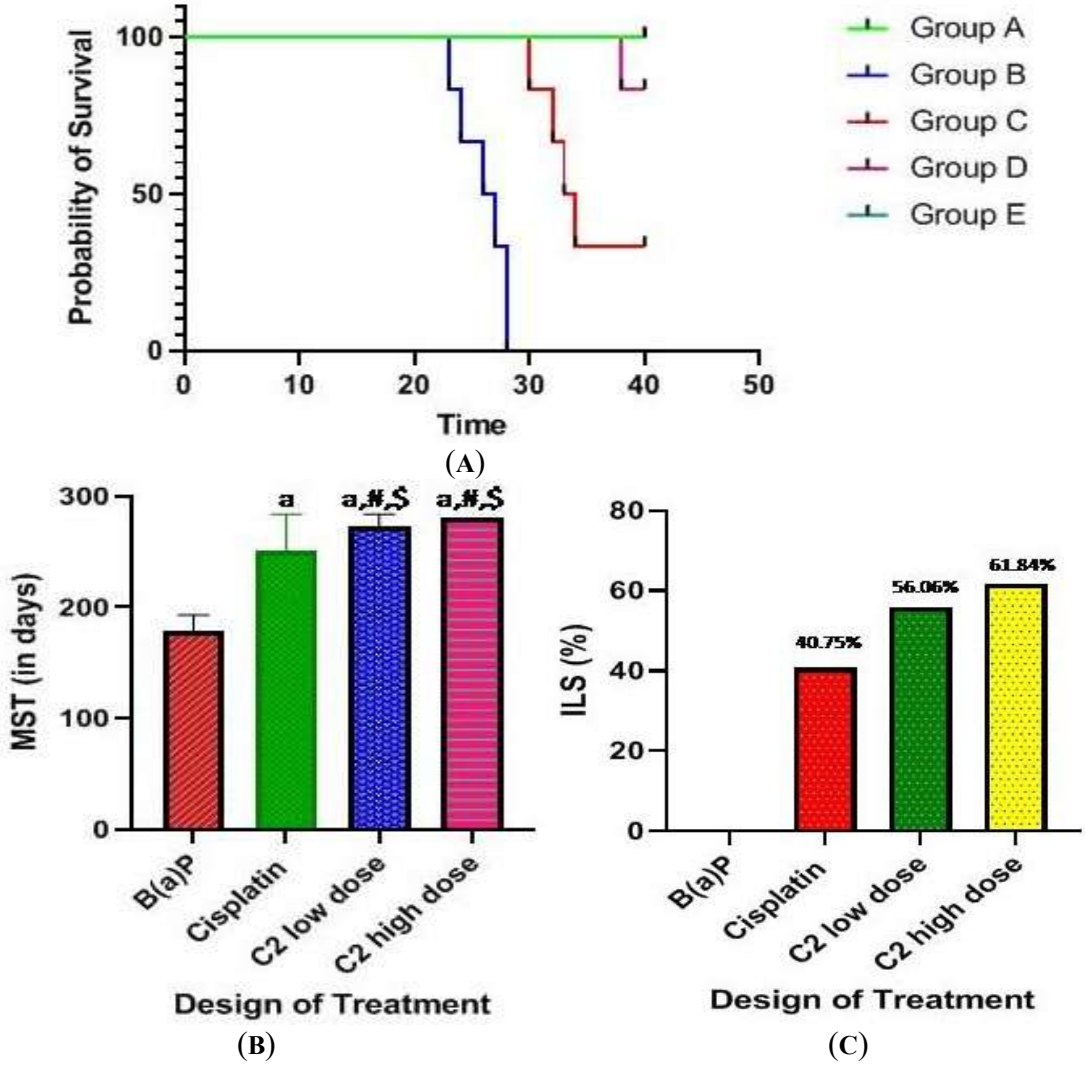


Figure 7: Percent survival of B(a)P induced tumor bearing mice as control and B(a)P induced tumor bearing treated mice with cobalt complex at dose 6.08mg/kg B.W., 12.17 mg/kg B.W and cisplatin were analyzed by Kaplan-Meier survival curve. The percent survival vs. Days graph was plotted and analyzed using Prism 9 software. Statistical significance was determined by one-way ANOVA followed by log-rank(Mantel-Cox)test (B) The median survival days after treatment with cobalt complex (6.08 and 12.17 mg/kg) are shown in percentage (%), [a]p<0.0001 when compared with B(a)P control group,#=no significance when compared with cisplatin group and ,$=no significance when compared between low and high doses groups of complex C2 (C) % increase in lifespan.

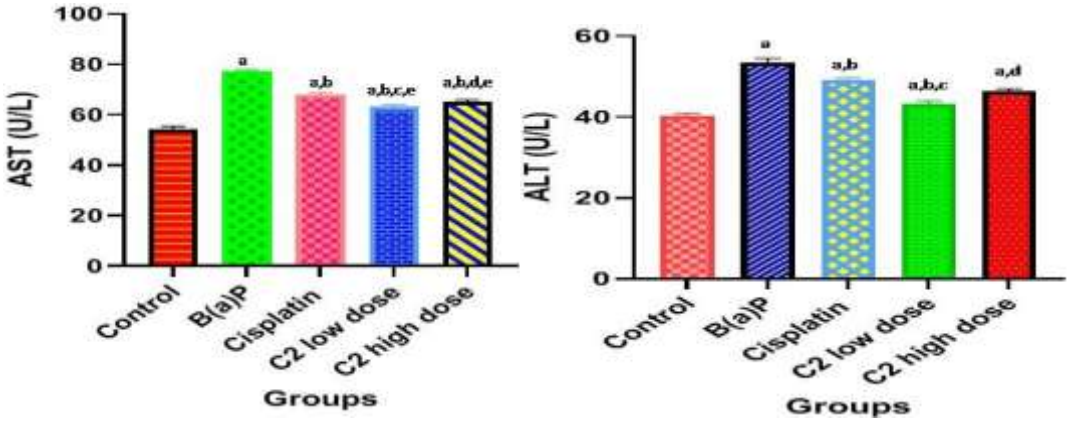
The Kaplan–Meier survival analysis for B(a)P, Cisplatin-treated (Figure 7), and Cobalt complex (C2) low and high dose groups indicated a significant increase in survival days for B(a)P-induced tumor-bearing mice treated with the cobalt complex. Specifically, treatment with C2 in low dose (6.08 mg/kg) and high dose (12.17 mg/kg) resulted in remarkable survival rate enhancements, achieving increases in lifespan by 56.06% and 61.84%, respectively, compared to a 40.75% increase with cisplatin. Median survival days also improved with C2 treatment, recorded at 46.11 and 46.66 days for low and high doses, in contrast to 35.64 days for the cisplatin group. The survival data demonstrated an 83% survival in the C2 low dose group and a 100% survival in the high dose group after 40 weeks. In contrast, the B(a)P-exposed group exhibited a median survival of 26.5 weeks, with C2 treatment demonstrating significant improvement ($p<0.0001$) and only one death reported in the C2 low dose group, while no death reported in the C2 high dose by the conclusion of the experiment. Conversely, the cisplatin-treated group had a median survival of 33.5 weeks.

4.4 Biochemical Analysis of Serum

B(a)P-induced tumor-bearing mice showed a significant decrease in albumin and glucose levels with elevated ALT and AST levels compared with control mice ($p<0.0001$) (Figure 7 and 8). Treatment with low and high doses of C2 restored albumin and glucose levels while reducing ALT and AST levels, indicating improved systemic and hepatic function (table 4).

Table 4. Effect of metal complexes C2 low and high doses on serum biochemical parameters of mice bearing B(a)P induced tumor.

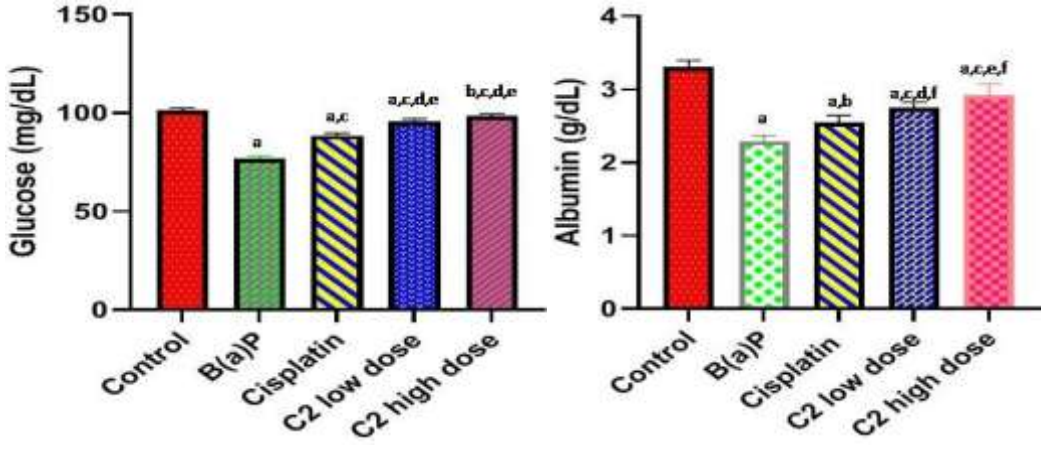
Groups	AST (U/L) SGOT	ALT (U/L) SGPT	Glucose (mg/dL)	Albumin (g/dl)
Normal control (vehicle)	54.32±1.03	40.26 ± 0.93	101.4 ± 1.14	3.313±0.08
Benzopyrene treated	77.28±0.68	53.56±1.01	76.90 ±1.40	2.297±0.07
Benzopyrene+Cisplatin	67.82 ±1.58	49.02±0.82	88.60±1.22	2.543±0.10
Benzopyrene+complex C2 low dose	63±4	43.15±0.82	95.94±1.10	2.632±0.08
Benzopyrene+complex C2 high dose	65±4	47.10±1.30	98.70±0.91	2.925±0.07



(A) (B)

Fig.7. Effect on various biochemical parameters level in different groups: (A) AST and (B) ALT level. Group 1 shows effect in vehicle control group, Group 2 shows effect after treatments with Benzopyrene only, Group 3 shows effect of standard drug Cisplatin on mice treated with Benzopyrene, Group 4 shows effect of Compound C2 low dose on mice treated with Benzopyrene and Group 5 shows effect of compound C2 high dose on mice treated with Benzopyrene. All the values are expressed as mean S.D. for six mice in each group. Statistical significance was determined by one-way ANOVA followed by Tukey post hoc test. (A) $^{a}p<0.0001$ when compared with normal control group, $^{b}p<0.0001$ when compared with B(a)P control group, $^{c}p<0.0001$ when compared with B(a)P control group, $^{d}p<0.001$ when compared with B(a)P control group, and $^{d}p<0.05$ when compared between C2 low and high dose groups. (B) $^{a}p<0.0001$ when compared with normal control group, $^{b}p<0.0001$ when compared with B(a)P control group, $cp<0.0001$ when compared with B(a)P control group, $^{d}p<0.0001$ when compared with B(a)P control group.

Cancer-induced metabolic alterations, including increased glucose utilization and reduced glucose synthesis [16, 17], may account for the decreased serum glucose and albumin levels in B[a]P-induced tumor-bearing mice[18,19]. Treatment with C2 restored glucose, albumin, ALT, and AST levels toward normal values compared with untreated tumor-bearing mice, indicating improved metabolic and hepatic function.



(a) GLUCOSE **(b) ALBUMIN**

Fig.8. Effect on various biochemical parameters level in different groups: (a) Glucose and (b) Albumin level. Group 1 shows effect in vehicle control group, Group 2 shows effect after treatments with Benzopyrene only, Group 3 shows effect of standard drug Cisplatin on mice treated with Benzopyrene, Group 4 shows effect of Compound C2 low dose on mice

treated with Benzopyrene and Group 5 shows effect of compound C2 high dose on mice treated with Benzopyrene. All the values are expressed as mean S.D. for six mice in each group. Statistical significance was determined by one-way ANOVA followed by Tukey post hoc test. (A) $^{a}p<0.0001$ when compared with normal control group, $^{b}p<0.01$ when compared with B(a)P control group, $^{c}p<0.0001$ when compared with cisplatin control group, $^{d}p<0.0001$ when compared with cisplatin control group, and #p< 0.01 when compared between C2 low and high dose groups. (B) $^{a}p<0.0001$ when compared with normal control group, $^{b}p<0.01$ when compared with B(a)P control group, $^{c}p<0.0001$ when compared with B(a)P control group, $^{d}p<0.05$ when compared with cisplatin group, and $^{d}p<0.0001$ when compared with cisplatin group, $^{d}p<0.05$ when compared with cisplatin group and $^{e}p<0.0001$ when compared with cisplatin group and $^{f}p<0.05$ when compared between C2 low and high dose groups.

4.7 Results of Haematological parameters

Hematological parameters of tumor-bearing mice after 16 weeks were found to be significantly altered from normal group. The administration of Benzopyrne leads to a significant decrease in hemoglobin, RBC and lymphocytes in tumor bearing animals, accompanied by a notable rise in the total number of white blood cells (W.B.Cs) and the percentages of lymphocytes, neutrophils, and monocytes. At the same treatment interval, complexes C2 at the dose of 6.08 mg/kg and 12.17 mg/kg changed these altered parameters significantly to near normal (Table 5).

Table 5: Effect of metal complexes C2 low and high doses on hematological parameters of mice bearing B(a)P induced tumor.

Parametres	Normal control	Benzopyrene	Benzopyrene +Cisplatin	B(a)P+ C2lowdose	B(a)P+C2high dose
RBC (U/L)	4.483±0.12	3.27±0.22	3.8±0.14	4.342±0.5	4.3±0.15
WBC (U/L)	6.24 ± 0.32	13.65±0.36	9.62±0.25	8.00±0.3	7.3±0.49
Haemoglobin(g/dL)	14.15 ± 0.28	6.593±0.25	11.05±0.64	12.29±0.37	13.21±0.84
Lymphocytes(%)	65.33 ±2.16	44.5±1.05	53.5±1.87	56.67±2.41	61.5±1.05
Neutrophils (%)	25.59±1.02	18.83±1.47	20.5±1.05	21.67±2.07	23.33±1.63
Eosinophils	1.25±0.06	0.53±0.20	0.8±0.14	1.05±0.19	1.2±0.21
Monocytes (%)	2.84±0.02	0.94±0.11	1.73±0.23	1.95±0.23	2.36±0.42
Platelets(×103/µL)	309×103± 1.64	300×103±1.08	306×103±0.84	310×103±0.3	321×103± 0.65

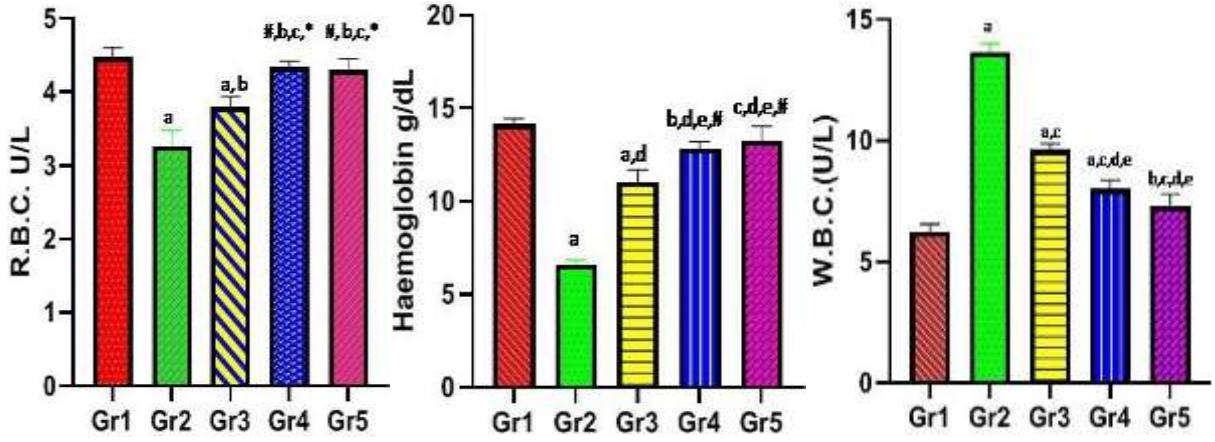


Fig 9: Effects on RBC, haemoglobin and WBC levels in different groups: Group 1 shows markers level in vehicle control group, Group 2 shows markers level after treatments with Benzopyrene only, Group 3 shows effect of standard drug Cisplatin on markers level in mice treated with Benzopyrene, Group 4 shows effect of Compound C2 low dose and Group 5 shows effect of compound C2 high dose on markers level in mice treated with Benzopyrene. All the values are expressed as mean S.D. for six mice in each group. Statistical significance was determined by one-way ANOVA followed by Tukey post hoc test; where (A) For R.B.C., $^{a}p<0.0001$ when compared with normal control group, $^{b}p<0.0001$ when compared with B(a)P control group, $^{c}p<0.0001$ when compared with cisplatin group and *= no significance when treated compounds C2 low and high dose were compared with each other (B) For Haemoglobin, $^{a}p<0.0001$ when compared with normal control group, $^{b}p<0.01$ when compared with normal control group, $^{c}p<0.05$ when compared with normal control group, $^{d}p<0.0001$ when compared with B(a)P control group $^{e}p<0.0001$ when compared with cisplatin group and # = no significance when treated compounds C2 low and high dose were compared with each other (C) For W.B.C, $^{a}p<0.0001$ when compared with normal control group, $^{b}p<0.001$ compared with normal control group, $^{c}p<0.0001$ when compared with B(a)P control group is compared with normal control group, $^{d}p<0.0001$ when compared with cisplatin group and $^{b}p<0.05$ treated compounds C2 low and high dose were compared with each other.

As a result, treatment with C2 low and C2 high doses, the tumor growth was inhibited. The above results indicated that the complexes C2 with both low and high doses have high antitumor efficacy and good safety, and it can be used as a potential antitumor candidate.

5. DISCUSSION

In summary, the potential anti-cancer activity of cobalt metal complex derived from β-diketones was investigated against Benzopyrene induced tumor bearing mice. A significant reduction in body weight accompanied by an increase in lung weight is a well-recognized hallmark of Benzo[a]pyrene (B(a)P)-induced lung carcinogenesis [11]. In the present study, B(a)P-treated mice exhibited a marked decline in body weight compared with the normal control group, which is consistent with previous reports. This loss of body weight is likely attributable to cancer-associated cachexia, anorexia, metabolic alterations, and impaired nutrient utilization, resulting in progressive depletion of adipose tissue and skeletal muscle mass [20]. Conversely, a significant increase in lung weight was observed in the B(a)P-treated group, which may be associated with enhanced tumor burden, the formation of multiple pulmonary nodules, inflammatory cell infiltration, edema, and tissue remodeling during tumor progression [21]. Treatment with the test compound significantly attenuated these changes, as evidenced by the restoration of body weight, reduction in lung weight, and decreased pulmonary nodule incidence compared with the B(a)P control group. These findings indicate that the compound exerts a protective effect against B(a)P-induced lung carcinogenesis, potentially through inhibition of tumor growth and mitigation of inflammation-associated pathological alterations in lung tissue. Histopathological examination of lung tissues from B(a)P-induced tumor-bearing mice revealed pronounced pathological alterations characteristic of lung carcinogenesis. The B(a)P-treated group exhibited a marked increase in hyperchromatic nuclei within the alveolar epithelial cells, extensive proliferation of the alveolar epithelium, disruption of normal alveolar architecture, and loss of normal pulmonary histoarchitecture, which are consistent with previous reports on B(a)P-induced lung tumorigenesis [22]. These histopathological abnormalities are likely attributable to excessive generation of reactive oxygen species and oxidative stress induced by B(a)P metabolism, leading to cellular damage, uncontrolled proliferation, and neoplastic transformation. In contrast, treatment with the test compound markedly attenuated these pathological changes, as evidenced by restoration of normal alveolar architecture, reduced epithelial hyperplasia, and a significant decrease in the number of hyperchromatic cells. The observed histological improvement may be attributed to the antioxidant and cytoprotective properties of the compound, which mitigate oxidative stress and preserve normal lung tissue integrity. Biochemical and haematological examination of the tested complex C2 increases albumin and glucose levels and decreases ALT and AST levels relative to tumor-bearing mice induced with Benzopyrene and restored altered haematological parameters significantly to near normal. These findings confirms the biochemical and haematological results of the present study and further demonstrate the protective potential of the compound against B(a)P-induced pulmonary carcinogenesis.

CONCLUSION

In conclusion, the present study reports for the first time that Co (II) complexes of β-diketonate derivatives prevents B(a)P-induced lung carcinogenesis in Swiss albino mice by significantly reducing the spread of Benzopyrene induced tumors, with an increase in the life span and survival time. Hence, $Co(dpq)_2(acac)_2$ may develop as a chemotherapeutic candidate with further detailed research to reduce the risk of human lung cancer.

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Conflicts of Interest

The authors declare no conflicts of interest.

REFERENCES

1. Bray F, Laversanne M, Sung H, Sung H., Ferlay J., Siegel R.L., Soerjomataram I., Jemal A., Global cancer statistics 2024: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2024;74(3):229–263. https://doi.org/10.3322/caac.21834.
2. S. Pilleron, E. Soto-Perez-de-Celis, J. Vignat, J. Ferlay, I. Soerjomataram, F. Bray, D. Sarfati, Int.J.Cancer 148(2021) 601-608.https://doi.org/10.1002/ijc.33232
3. Hogan N. J., Shiu K.K., Khan K., Challenges in the treatment of gastric cancer in the older patient, Cancer Treat Rev. 85 (2020), 101980.
4. Bouvier A.M., Crehange G., Azelie C., Cheynel N., Jouve J.L., Bedenne L., J. Faivre, C. Lepage, P. Maingon, Adjuvant treatments for gastric cancer: from practice guidelines to clinical practice, Digest. 2014 Jan; 46(1):72-5.
5. D. L. Ma, H. Z. He, K. H. Leung, D. S. H. Chan, C. H.Leung, Angew. 52 (2013) 7666-7682.https://doi.org/10.1002/anie.201208414
6. P. Jia, R. Ouyang,P. Cao, X. Tong, X. Zhou, T. Lei, S. Zhou, J.Coord Chem. 70 (2017) 2175-2201.https://doi.org/10.1080/00958972.2017.1349313
7. B. Rosenberg, L. Van Camp, T. Krigas, Nature. 205 (1965) 698–699.https://doi.org/10.1038/205698a0. 818
8. C. Rancoule, J.-B. Guy, A. Vallard, M. Ben Mrad, A. Rehailia, N. Magné, Les 50 ans du cisplatine, Bull. Cancer (Paris). 104 (2017) 167–176. https://doi.org/10.1016/j.bulcan.2016.11.011. 821
9. Gogoi A., Sandilya P.B., Saikia K.K., Evaluation of Bioessential Transition Metal Complexes of β-diketonate derivatives as Promising Anticancer Chemotherapeutics .International Journal of Aquatic research and environmental Studies, 6(1s) (2026) 01-25.

10. Shah, A.H.; Qureshi, S.; Tariq, M.; Ageel, A.M. Toxicity studies on six plants used in the traditional Arab system of medicine. Phytother. Res. 1989, 3, 25–29
11. Kasala ER, Bodduluru LN, Barua CC, Madhana RM, Dahiaya, Budhania MK et al. Chemopreventive effect of chrysin, a dietary flavone against benzo(a)pyrene induced lung carcinogenesis in Swiss albino mice. September 6, 2015. 10.1016/ j.pharep.2015.08.014
12. Dolai N Karmakar, I Suresh Kumar, RB Kar, B Bala, A Haldar, PK (2012) Evaluation of Antitumor activity and in vivo antioxidant status of Anthocephalus cadamba on Ehrlich ascites carcinoma treated mice. Journal of ethnopharmacology 142(3): 865-870.
13. Sur P Ganguly, DK (1994) Tea plant root extract (TRE) as ann antineoplastic agent. Planta medica 60 (02): 106-109
14. Gupta M. Mazumder, UK Rath, N, Mukhopadhyay DK (2000) Antitumor activity of methanolic extract of Cassia fistula L. seed aginst Ehrlich Ascites Carcinoma. Journal of ethnopharmacology 72 (1): 151-156.
15. Boorman G.A., Pathology of the Fischer Rat: Reference and Atlas, Academic Press, 1990.
16. Oscar C., Biopharmacological studies on certain new anticancer drugs. J. Biochem. 1988, 110, 701-701.
17. Tagmizyan, WA. Metabolism of impaired glucose in patients with neoplasia. Am. Biol. 1990, 2, 53-58.
18. Silverstein, H., Dervot K., Oscar D. Studies on carbohydrate metabolism and different types of tumors bearing animals. Lancet 1988, 22, 40-45.
19. Kind P.R.N., Gordon M., Laverick M., Nias A.H., Slavin B.M. The effect of C3H mouse mammary tumor on the levels of serum and urine analysis in vivo. Br. J. Cancer 1985, 52, 607-612.
20. Tessitore L., Costelli P., Baccino F.M., Pharmacological interference with tissue hypercatabolism in tumour-bearing rats, Biochem. J. 299 (1994) 71–78.
21. Magesh V., Singh J.P., Selvendiran K., Ekambaram G., Sakthisekaran D., Antitumour activity of crocetin in accordance to tumor incidence, antioxidant status, drug metabolizing enzymes and histopathological studies, Mol. Cell. Biochem. 287 (1–2) (2006) 127–135.
22. L.N. Bodduluru, E.R. Kasala, C.C. Barua, M.R. Madhana, I. Hussain, P. Borah, Naringenin ameliorates inflammation and cell proliferation in benzo(a)pyrene induced pulmonary carcinogenesis by modulating CYP1A1, NF-kB and PCNA expression, Int. Immunopharmacol. 30 (2016) 102–110.