International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

IJFBS
International Journal of Fauna And Biological Studies

E-ISSN 2347-2677
P-ISSN 2394-0522
www.faunajournal.com
IJFBS 2021; 8(4): 31-34
Received: 17-05-2021
Accepted: 19-06-2021

Preethy John
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

Nisha AR
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

Mukul CG
M.VSc. Scholar, Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

Corresponding Author:
Preethy John
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

Ameliorative effect of baicalein nanoparticles on body weight loss and tumour growth in Daltons Lymphoma Ascites (DLA) cell induced tumour mice model

Preethy John, Nisha AR and Mukul CG

DOI: https://doi.org/10.22271/23940522.2021.v8.i4a.842

Abstract
Objective: Baicalein has demonstrated a wide variety of biological activities including anticancer property. The present study was aimed to evaluate effect of silver nanoparticles synthesised using baicalein on body weight and tumour weight in Daltons Lymphoma Ascites (DLA) cell induced solid tumour in Swiss albino mice.
Methods: Tumour positive animals were randomly allocated to seven groups of six animals. Group I served as normal control Group II as tumour control, Group III was administered with 5-Fluorouracil (5-FU) at 20 mg/kg orally. Group IV and V received baicalein nanoparticles (B-AgNPs) at 50 mg/kg and 100 mg/kg respectively. Group VI received pure compound, baicalein (BCLN) at 100 mg/kg. Chemically synthesised nanoparticles (C-AgNPs) at 100 mg/kg was administered to Group VII. All the treatments were given orally for 10 days. Body weight was recorded on days zero, five and 11. All the animals were sacrificed after 10 days treatment and collected tumour mass to assess tumour weight and ratio of tumour weight to body weight.
Result: All the groups except normal control showed reduction in body weight but the weight loss was significantly less compared to C-AgNPs treated group and tumour control group. A significant dose dependant reduction in tumour weight and ratio of body weight to tumour was noticed in animals treated with B-AgNPs at both high and low doses compared to other groups.
Conclusion: The oral administration of B-AgNPs @100 mg/kg was found to have a significant ameliorative effect on body weight loss and tumour growth in Daltons Lymphoma Ascites (DLA) cell induced tumour mice model. In the present study it was also found that biosynthesised nanoparticles (B-AgNPs) produced more activity than the pure compound (BCLN) from which they were synthesised.

Keywords: Dalton's lymphoma**,** baicalein nanoparticles

Introduction
Cancer is the second leading cause of death in the world after the cardiac diseases. Currently, the main treatments for cancer are chemotherapy, radiotherapy and surgery, among which chemotherapy is routinely used for cancer treatment. However, most of the clinically used synthetic chemotherapeutic agents exhibit severe normal tissue toxicity and cause undesirable side effects in the patients receiving them. Therefore, there is a need to find alternative drugs, which at non-toxic doses are highly effective and affordable. The secondary metabolites produced from plants were investigated for their anticancer activities and many plant-based products including alkaloids, flavonoids etc. have shown promising anti-cancer potential alone or in combination with conventional anticancer drugs. With the success of these compounds as effective and safe drugs for cancer treatment, new technologies are emerging to develop the area further. The application of various nanotechnologies referred to as cancer Nano-medicine is aimed to enhance anticancer activities of plant-derived drugs by controlling the release of the compound and investigating new methods for administration to have more effective cancer treatment.
Baicalein (5, 6, 7- trihydroxyflavone), a flavone originally isolated from the roots of *Scutellaria baicalensis* and *Scutellaria lateriflora* is also reported to be present in *Oroxylum indicum* (Palakapayyani). Baicalein had been documented to have efficient pharmacological activities including anticancer activity *in vitro* and *in vivo.* The present study was aimed to evaluate effect of silver nanoparticles synthesised using baicalein on body weight and tumour weight in Daltons Lymphoma Ascites (DLA) cell induced solid tumour in Swiss albino mice.

Materials and Methods

Baicalein nanoparticles were synthesised as per method of Sahu *et al*., 2016 [1]. Silver nanoparticles were chemically synthesised by Turkevich method (1951) [2]. Prepared nanoparticles were characterised.

Solid tumour induction and treatment

Viable DLA cells (1×10^6cells/mouse) were injected subcutaneously into the right hind limb of Swiss albino mice. After 10 days, tumour development was assessed by determining tumour volume. Tumour positive animals were used for further study.

Forty two tumour positive mice were selected and randomly allocated into nine groups with six animals in each group. Normal control (Group I) also comprised of six animals. The treatments were as follows.

Group	Treatment
I	Normal control
II	Tumour control without any treatment
III	Tumour positive animals treated with standard drug 5-fluorouracil (20 mg/kg body weight) orally
IV	Tumour positive animals treated @ 50 mg/kg of baicalein nanoparticle
V	Tumour positive animals treated @ 100 mg/kg of baicalein nanoparticle
VI	Tumour positive animals treated @ 100mg/kg of baicalein
VII	Tumour positive animals treated @ 100mg/kg of silver nanoparticles

The starting day of oral administration of the reference drug/test substance was taken as day zero. Body weight was recorded on days zero, five and 11. Animals were sacrificed and tumour masses were collected on day 11 to assess tumour weight, ratio of tumour weight to body weight.

Body weight

The body weight of the mice was recorded on day zero (Initial body weight), days five and 11 (final body weight) and body weight loss was calculated as follows:

$$\text{Per cent body weight loss} = \frac{\text{Initial body weight -Final body weight x100}}{\text{Initial body weight}}$$

Tumour weight

After sacrificing the animals, the tumour mass was collected and the tumour weight of mice was recorded.

Ratio of tumour weight to body weight

The ratio of tumour weight to body weight was calculated by dividing tumour weight by body weight

Result

Body weight

The effect of treatment with test compounds for 10 days on body weight is furnished in Table 1 and Fig.1. The mean body weight of all animals except group I animals was found to be decreased on days five and 11 when compared to day zero. Group I showed a significant ($p< 0.05$) increase in mean body weight from 23.27 ± 0.45 g on day five to 24.31 ± 0.49 g on day 11.

The per cent of body weight loss after treatment with test compounds for 10 days is depicted in table 2 and Fig. 2. Maximum loss in mean body weight was observed for untreated group (25.42 ± 0.98%) and C-AgNP treated groups (22.98 ± 1.78%) followed by baicalein (17.95 ± 1.04%). B-AgNP treated groups showed a significant ($p< 0.05$) reduction in body weight loss in a dose dependent manner ie., B-AgNP at low dose showed 12.97 ± 1.21% reduction while at high dose B-AgNP showed 4.8 ± 0.53% reduction. The standard drug, 5-FU showed 9.46 ± 0.52% reduction in body weight loss.

Table 1: Effect of different test compounds on body weight of mice with DLA induced solid tumour

Groups	Day 0	Day 5	Day 11	F-value (P-value)
I	22.34 ± 0.42^{C}	23.27 ± 0.45^{aB}	24.31 ± 0.49^{aA}	193.10** (<0.001)
II	23.28 ± 0.75^{A}	20.35 ± 0.8^{cB}	17.38 ± 0.67^{dC}	474.801** (<0.001)
III	22.05 ± 0.62^{A}	20.99 ± 0.64^{bcB}	19.98 ± 0.65^{cC}	371.368** (<0.001)
IV	22.64 ± 0.79^{A}	20.93 ± 0.82^{bcB}	19.74 ± 0.94^{cC}	134.378** (<0.001)
V	23.28 ± 0.85^{A}	22.74 ± 0.92^{abB}	22.18 ± 0.92^{abC}	88.404** (<0.001)
VI	23.5 ± 0.89^{A}	21.41 ± 0.91^{abcB}	19.33 ± 0.96^{cdC}	722.86** (<0.001)
VII	22.51 ± 0.58^{A}	19.9 ± 0.55^{cB}	17.37 ± 0.8^{dC}	174.76** (<0.001)
F-value (P-value)	0.714^{ns} (0.693)	3.339** (0.003)	10.831** (<0.001)	

Values expressed as mean ± SEM (n=6). **Significant at 0.01 level; ns- non-significant

Means bearing different small letter as superscript differ significantly within a column; Means bearing different capital letter as superscript differ significantly within a row.

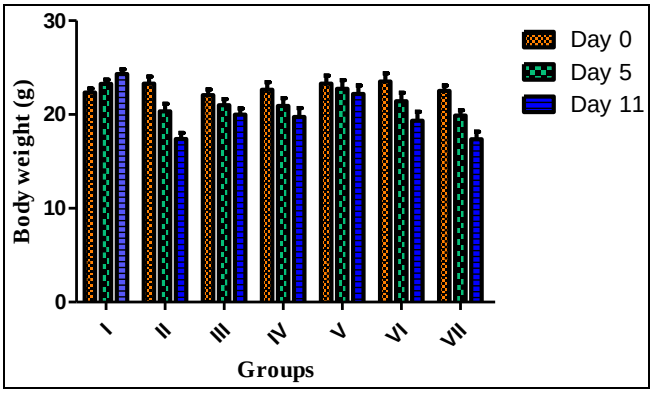

Fig 1: Effect on body weight of mice with DLA induced solid tumour of different test compounds after 10 days treatment with various test compounds

Table 2: Effect of different test compounds on per cent body weight loss of mice with DLA induced solid tumour

Group	Body Weight Loss (%)
II	25.42 ± 0.98[a]
III	9.46 ± 0.52[d]
IV	12.97 ± 1.21[c]
V	4.8 ± 0.53[e]
VI	17.95 ± 1.04[b]
VII	22.98 ± 1.78[a]
F-value (P-value)	95.116** (<0.001)

Values expressed as mean ± SEM (n=6). Means bearing different superscripts differ significantly within a column

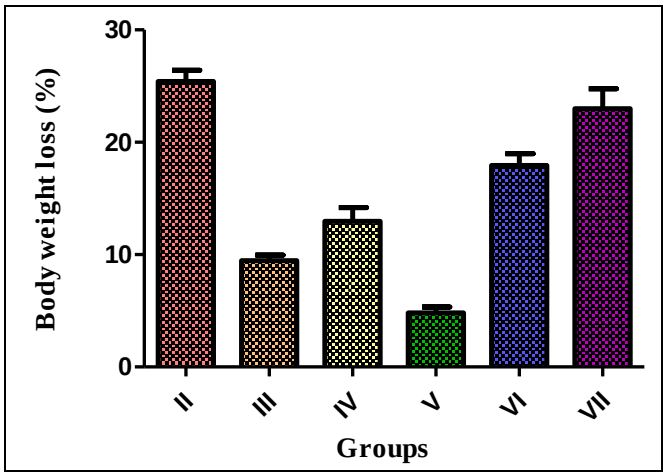

Fig 2: Per cent body weight loss after 10 days treatment with various test compounds in different groups

Tumour weight

The effect of different test compounds on DLA induced solid tumour weight is depicted in Table 3 and Figures 3 and 4. The mean tumour weight was found maximum in group II (1.63 ± 0.05 g) compared to all the other groups. Next highest value was shown by group VII (0.9 ± 0.01 g) followed by group VI (0.75 ± 0.02 g). A dose dependant decrease was noted in group IV (0.66 ± 0.03 g) and group V (0.54 ± 0.02 g) animals treated with B-AgNP at low dose and high dose respectively. The mean tumour weight of group III animals treated with 5-FU was 0.58 ± 0.03 g which was comparable with group V treated with B-AgNP at high dose.

A dose dependant reduction was noticed in B-AgNP treated groups which were 59.33 ± 2.48% (low dose) and 66.82 ± 2.02% (high dose) and were comparable to 5-FU treated group III (64.4 ± 2.46%). Group VII treated with C-AgNPs showed least reduction which was 44.75 ± 2.12%.

Table 3: Effect of different test compounds against DLA induced solid tumour weight and ratio of tumour weight to body weight in mice.

Group	Tumour weight	Reduction in tumour weight (%)	Tumour weight to body weight ratio
II	1.63 ± 0.05[a]	-	0.09 ± 0.004[a]
III	0.58 ± 0.03[e]	64.4 ± 2.46[cd]	0.029 ± 0.002[de]
IV	0.66 ± 0.03[d]	59.33 ± 2.48[de]	0.034 ± 0.002[cd]
V	0.54 ± 0.02[e]	66.82 ± 2.02[c]	0.024 ± 0.002[ef]
VI	0.75 ± 0.02[c]	53.92 ± 1.91[e]	0.04 ± 0.002[c]
VII	0.90 ± 0.01[b]	44.75 ± 2.12[f]	0.05 ± 0.002[b]
F-value (P-value)	266.288** (<0.001)	52.683** (<0.001)	150.71** (<0.001)

Values expressed as mean ± SEM (n=6). Means bearing different superscript differ significantly within a column

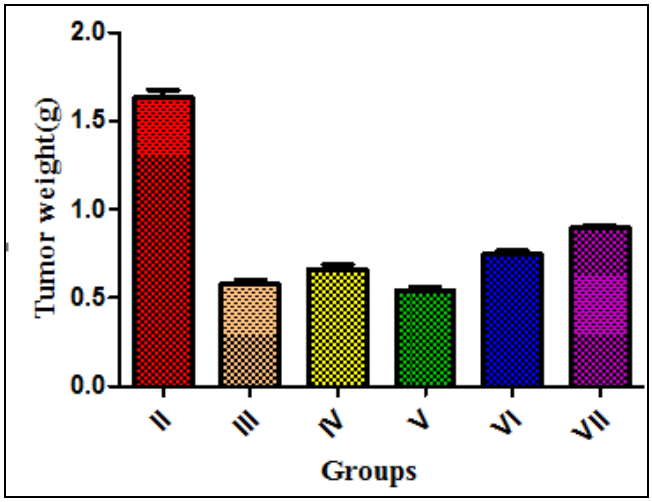

Fig 3: Effect of different test compounds on DLA induced solid tumour weight

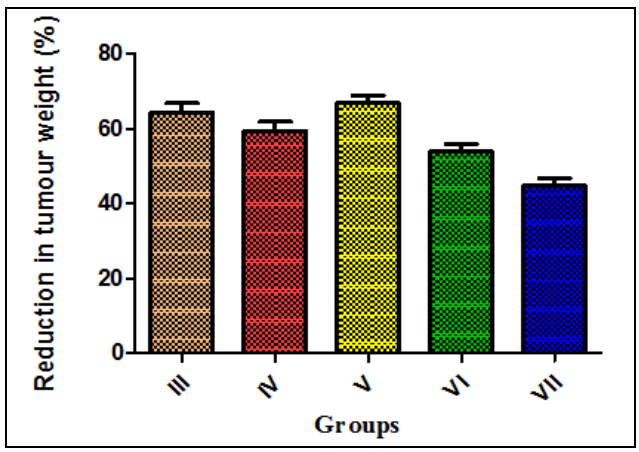

Fig 4: Per cent reduction in tumour weight after 10 days of treatment with test compounds

Ratio of tumour weight to body weight

The result is presented in table 3 and figure 5. Group II exhibited a significantly (p< 0.05) high tumour weight to body weight ratio (0.094±0.004) followed by group X (0.052±0.002). A decreasing mean tumour weight to body weight ratio was noticed in the order 0.039±0.002 (group VI), 0.034±0.002 (group IV), 0.029±0.002 (group III), 0.024±0.002 (group V).

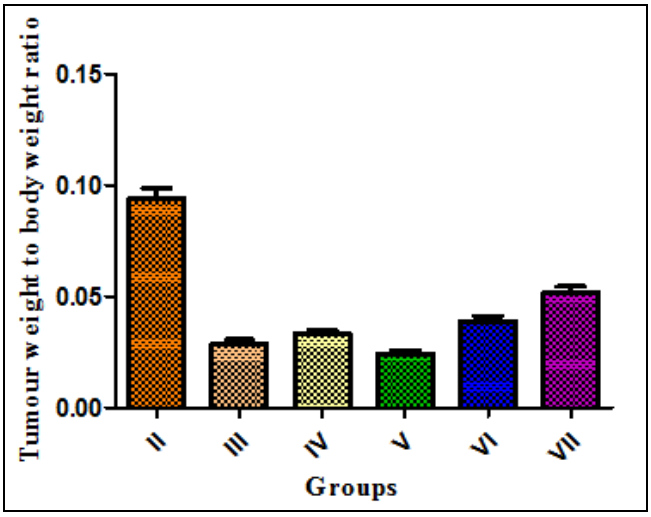

Fig 5: Ratio of tumour weight to body weight after 10 days of treatment with test compounds

Discussion

Body weight

In the present study the mean body weight of animals of different groups was analysed on days zero, five and 11. There was a reduction in mean body weight of all the animals on days five and 11 except tumour negative normal control animals (G I) where mean body weight increased significantly ($p< 0.05$) on days five and 11 as compared with their initial body weight on day zero. In tumour positive control group (G II) and C-AgNP treated group (G VII), there was drastic fall in mean body weight. The result indicated that presence of tumour affected the normal growth and weight gain of the animals. In B-AgNP treated groups (G IV and GV), there was a dose mean body weight dependant decrease in noticed over a period of 11 days. The mean body weight of B-AgNP @ low dose treated group IV was comparable to the group treated with the standard anticancer drug 5-FU (III) where the change was not statistically significant. Dhamija *et al*. (2013) [3] observed a drastic fall in body weight in mice with DLA cell induced solid tumour treated with higher dose of alcoholic extract of *Premna herbacea* and its ethyl acetate fraction compared to zero day reading. But in the present study, even though the presence of tumour affected the weight gain of animals, may be due to the growth promoter activity of BCLN as reported by Zhou *et al*. (2019) [4], there was not a drastic fall in body weight as observed in G II and G VII.

Tumour weight

BCLN (G VI), B-AgNP at 100 mg/kg (G V) and B-AgNP at 50 mg/kg (G IV) also produced a reduction in tumour weight which were 53.92 ± 1.91%, 59.33 ± 2.48% and 66.82 ± 2.02% respectively. This result is in agreement with studies performed by Chen *et al*. (2013) [5] who reported the significant inhibition of the growth of bladder cancer cells in mice treated with BCLN and Wang *et al*., 2015 [6] who reported the significant inhibition of growth of colorectal cancer cells (HCT-116) induced tumour in mice that received BCLN. The reduction shown by B-AgNPs was dose dependant and comparable to that produced by 5-FU (64.40±2.46%). Among the treated groups, G VII animals treated with C-AgNP showed least reduction in tumour weight (44.75±2.12%).

Ratio of tumour weight to body weight

In tumour positive control group (G II) and C-AgNP treated group (G VII), there was drastic fall in mean body weight but the mean tumour weight increased considerably and hence the ratio of tumour weight to body weight also increased. In B-AgNP treated groups (G IV and GV), there was a dose dependant decrease in mean body weight and a slight reduction in mean tumour weight which reflected as the decreased ratio of tumour weight to body weight. This is suggestive of the tumouricidal action of BCLN. Ratio was comparable to the group treated with the standard anticancer drug 5- FU though the change was statistically not significant. 100mg/kg dose rate of B-AgNP was found to have a more pronounced effect than respective lower dose 50 mg/kg groups which is suggestive of a dose dependent inhibitory effect of biosynthesised nanoparticles on tumour growth.

Conclusions

Biosynthesised nanoparticles (B-AgNPs) produced more activity than the pure compound (BCLN) from which they were synthesised. The present finding that the biosynthesised nanoparticles possessed tumour growth inhibitory activity is very encouraging and may be due to improved bioavailability.

References

1. Sahu N, Soni D, Chandrashekhar B, Satpute DB, Saravanadevi S, Sarangi BK *et al*. Synthesis of silver nanoparticles using flavonoids: hesperidin, Naringin and Diosmin, and their antibacterial effects and cytotoxicity. Nano Lett 2016;6(3):173-181.
2. Turkevich J, Stevenson PC, Hillier J. A study of the nucleation and growth processes in the synthesis of colloidal gold. Discuss. Faraday Soc 1951;11:55-75.
3. Dhamija I, Kumar N, Manjula SN, Parihar V, Setty M, Pai KSR. Preliminary evaluation of *in vitro* cytotoxicity and *in vivo* antitumor activity of *Premna herbacea* Roxb. In Ehrlich ascites carcinoma model and Dalton's lymphoma ascites model. Exp. Toxicol. Pathol 2013;65:235-242.
4. Zhou Y, Mao S, Zhou M. Effect of the flavonoid baicalein as a feed additive on the growth performance, immunity and antioxidant capacity of broiler chickens. Poult Sci 2019;98(7):2790-2799.
5. Chen YJ, Wu CS, Shieh JJ, Wu JH, Chen HY, Chung TW *et al*. Baicalein triggers mitochondria-mediated apoptosis and enhances the antileukemic effect of vincristine in childhood Acute Lymphoblastic Leukemia CCRF-CEM cells. Evid. Based Complement. Alternate Med 2013, 1-19.
6. Wang C, Zhang C, Chen L, Anderson S, Lu F, Yuan C. Colon cancer Chemopreventive effects of baicalein, an active enteric micro biome metabolite from baicalin. Int. J. Oncol 2015;47:1749-1758.