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ANTITUMOUR ACTIVITY OF QUERCETIN-3-O- β -GLUCOSIDE ISOLATED FROM *CANSJERA RHEEDII* (OPILEACEA) AGAINST DALTON'S ASCITIC LYMPHOMA

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ABSTRACT

The antitumour activity of Quercetin-3-O-β-Glucoside (5, 7, 3', 4'-Tetrahydroxy-3-O-β-D-Glucopyranosyl flavone) isolated from aerial parts of Cansjera rheedii J.Gmelin (Opiliaceae) has been evaluated against Dalton's ascitic lymphoma (DAL) in swiss albino mice. A significant enhancement of mean survival times of Quercetin-3-O-β-Glucoside treated tumour bearing mice was found with respect to control group. Quercetin-3-O-β-Glucoside treatment was found to enhance peritoneal cell counts. When these Quercetin-3-O-β-Glucoside treated animals underwent i.p. inoculation with DAL cells, tumour cell growth was found to be inhibited. After 14 days of inoculation, Quercetin-3-O-β-Glucoside is able to reverse the changes in the haematological parameters, protein and PCV consequent to tumour inoculation.

KEYWORDS: Dalton's Ascitic Lymphoma, Quercetin-3-O-β-Glucoside, Mean Survival time, Haematological parameters, Cansjera rheedii.

INTRODUCTION

Cansjera rheedii J Gmelin (Opiliaceae) is a climbing shrub, sometimes armed, generally found in India through Malaya to Hong Kong and North Australia.[1-2] The tribes of Nilgiris in Tamil Nadu, India using the plant extract for the treatment of post-natal pain[3], intermittent fever[4] and poisonous bites and skin diseases.[5] In our earlier studies, the ethanol extract of aerial parts of *C.rheedii* has been reported to have hepatoprotective[6], cytotoxic[7], anthelmintic[8], anti-inflammatory and membrane stabilizing property[9], antipyretic[10], anti-nociceptive[11] and diuretic[12] activities. The safety of this plant has also been proved by studying acute and sub-acute toxicity studies.[13] The compounds such as 3, 4-dihydroxy cinnamic acid (Caffeic acid) **(I)**, 4-hydroxy 3-methoxy cinnamic acid (Ferulic acid) **(II)**, 3, 5, 7, 3', 4'-pentahydroxy flavone (Quercetin) **(III)**, 5, 7, 3', 4'-tetrahydroxy -3-O-β-D-glucopyranosyl flavones (Quercetin-3-O-β-glucoside) **(IV)** and 5, 7, 3', 4'-tetrahydroxy-3-O-(6-O-α-L-rhamnopyanosyl)-β-D-glucopyranosyl flavone (Quercetin-3-O-β-rutinoside (or) Rutin)(**V**). Structures of all these compounds were established by spectral and chemical methods.[14] This was the first report of the above 5 compounds from the plant. A variety of phenolic compounds have been shown to suppress carcinogen-induced mutagenesis and neoplasia in experimental animals.[15-18] Hence the present study is designed to find out the effect of Quercetin-3-O-β-glucoside (IV) isolated from aerial parts of *Cansjera rheedii* J.Gmelin (Opiliaceae) against Dalton's ascetic lymphoma.

MATERIAL AND METHODS

Extraction and isolation: The air dried and coarsely powdered aerial parts (1.0Kg) were extracted with boiling 95% ethanol (3 X 5l) and the extract was concentrated to about 250 ml. The insoluble green residue was removed by filtration and the soluble in the filtrate (150 ml) were fractioned into C_6H_6, Et_2O, EtOAc and EtCOMe. The C_6H_6 fraction after concentration yielded a pale yellow needle, recrystallized from MeOH and designated as compound **I** (910mg). The Et_2O concentrate was column chromatographed over sephadex LH-20 using MeOH. 35 fractions of 50ml each were collected. Fraction 4-29 gave colourless needles, recrystallized from MeOH and designated as compound **II** (800mg). The EtOAc concentrate was column chromatographed over Sephadex LH-20 using MeOH. 44 fractions of 50ml each where collected, fractions 7-32 gave yellow needles, recrystallized with MeOH and designated as Compound-**III** (1.1g). The EtCOMe concentrate was chromatographed on a column of Sephadex LH-20 using MeOH as eluent. 107 fractions of 50ml each were collected, fractions 6-35 deposited a homogenous yellow solid recrystallized from MeOH and designated as compound-**IV** (89mg). Fractions 36-98 gave a pale yellow homogenous solid, recrystallized from MeOH and were designated as compound-**V** (530mg).

Characterization of Compound-IV (5, 7, 3', 4'-tetrahydroxy -3-O-β-D-glucopyranosyl flavone)

Yellow powder, mp. 219.47° C gave yellow colour with alkalis, pink with Mg-HCl, Olive green with Fe^{3+} and positive Molisch's test.[19] It was purple under UV and yellow under UV/NH_3. R_f characteristic of flavonoid. Acidhydrolysis yielded an aglycone identified as quercetin and a sugar identified as D-glucose. The above two products are identified by co-PC with authentic sample of glucose. UV λ_{max} (MeOH): 256, 297sh, 356nm; (+NaOMe): 272, 324, 412nm ;(+CH_3COONa):268,299sh, 358,402nm; (+CH_3COONa /H_3BO_4): 268,299sh, 358,402nm; (+$AlCl_3$): 274,300sh, 425nm ;(+ $AlCl_3$/HCl): 268,298sh, 360,403nm. ^{1}H NMR (500MHz) DMSO-d_6 ; δ 12.57 (s, 1H, 5-OH); δ 10.56 (s, 1H, OH-7); δ 9.26(s, 1H, OH-4'); δ 9.12 (s, 1H, OH-3'); δ 7.53 (d, J= 2.2 Hz, 1H, H-2'); δ 7.52 (dd, J=8.8 & 2.2Hz, 1H, H-6'); δ 6.81 (d, J=8.8Hz, 1H, H-5'); δ 6.37 (d, J=2.2Hz, 1H, H-8); δ 6.16 (d, J=1.5 Hz, 1H, H-6) of aglycone; δ 5.40 (d, J=8.80 Hz, 1H, H-1''); δ 3.30 (d, J=8.8 Hz, 1H, H-2''); δ 3.19 (m,4H, H-3'', H-4'', H-6'' α); δ 3.05 (m, 1H, H-5''); δ 3.71 (dd, J= 10.3 Hz & 8.8 Hz, 1H, H-6''β) of glucose. ^{13}C NMR (500MHz) DMSO-d_6; assignment based on HSQC and HMBC, δ 156. 20 (s, C-2); δ 133.30 (s, C-3); δ 174.61 (s, C-4); δ 161.70 (s, C-5); δ 94.10 (d, C-6); δ 164.4 (s, C-7); δ 93.40 (d, C-8); δ 156.60 (s, C-9); δ 103.9 (s, C-10); δ 121.60 (s, C-1'); δ 115.71 (d, C-2'); δ 144.70 (s, C-3'); δ 149.40(s, C-4'); δ 116.48 (d, C-5'); δ 121.92 (d, C-6') of aglycone. δ 101.82 (d, C-1''); δ 76.58 (d, C-2''); δ 77.98 (d, C-3''); δ 76.99 (d, C-4''); δ 70.37 (d, C-5''); δ 61.20 (t, C-6'') of glucose. MS (+ve and -ve), *m/z* 487 $[M+Na]^+$, 325 $[M+Na-162]^+$, 463 $[M-H]^-$, 301 $[M-H-162]^-$. IR (KBr) cm^{-1}: 3365 br, 2921, 1657, 1608, 1502, 1450, 1365, 1303, 1198, 1058, 1010, 937, 809, 722, 655 and 592. Elemental analysis (%): C (54.30): H (4.26): O (41.44). Thus compound IV was identified as 5, 7, 3', 4'-tetrahydroxy-3-O-β-D-glucopyranosyl flavones[20-23] (Figure 1).

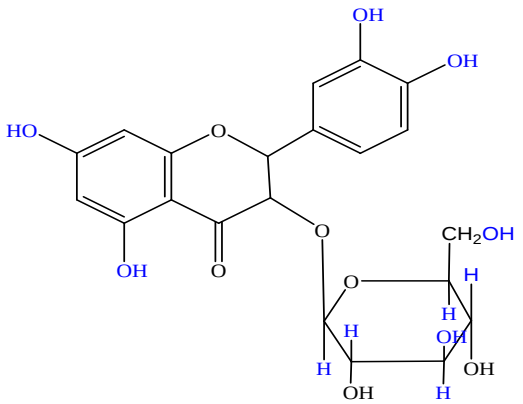

Figure. 1. Structure of 5, 7, 3', 4'-tetrahydroxy-3-O-β-D-glucopyranosyl flavone.

Animals: Swiss albino mice (24-28g) were used throughout the study. They were housed in standard microlon boxes and were given standard laboratory diet and water *ad libitum*.

Cells: Dalton's ascitic lymphoma (DAL) cells were obtained courtesy of the Cancer Reseach Centre (CRC), Adyar, Chennai and given by intraperitoneal transplantation of 10^6cells/mouse.[24-25]

Effect of Quercetin-3-O-β-glucoside on survival time[26]

Animals were inoculated with 10^6 cells/mouse on day 0 and treatment with Quercetin-3-O-β-glucoside started 24 hrs, after inoculation, at a dose of 50mg/kg/day intraperitoneally (group-A). The control group (group-B) was treated with the same volume of 0.9% sodium chloride solution. All treatments were carried out for 9 days. Mean survival times (MST) of each group, containing 8 mice were noted. The antitumour efficiency of Quercetin-3-O-β-glucoside was compared with control group using the following equation.[27]

Increase of life=MST of treated group x 100/MST of control group

Effect of Quercetin-3-O-β-glucoside on normal peritoneal cells[28]

Three groups of normal mice (n=5) were used for the strudy. One group was treated with 50mg/kg i.p., of Quercetin-3-O-β-glucoside and the second group received the same treatment for 2 consecutive days. The untreated third group was used as control. Peritoneal exudate cells were counted 24hr after treatment for each treated group and compared with those of the untreated group.

Effect of Quercetin-3-O-β-glucoside on haemotological parameters

In order to detect the influence of Quercetin-3-O-β-glucoside on the haemotological status of DAL bearing mice, comparison was made amongst three groups (n=5) of mice on the 14th day after inoculation. The three groups comprised (i) Tumour bearing mice (ii) tumour bearing mice treated with Quercetin-3-O-β-glucoside (50mg/kg/day i.p) for 9 days and (iii) normal mice. Blood was drawn from each mouse in the conventional way and the white blood cell count, red blood cell count, haemoglobin, protein, differential count and packed cellular volume were determined.[24-26] All the results were analyzed by analysis of variance.[29]

RESULTS

The effect of Quercetin-3-O-β-glucoside (5, 7, 3', 4'-tetrahydroxy -3-O-β-D-glucopyranosyl flavone)
on the survival of tumour bearing mice showed MST for the control group to be 19days, while it was 25 days and 29 days for the groups treated with Quercetin-3-O-β-glucoside (50mg/kg/day ip) and 5FU (20mg/kg/day ip) respectively. (Table-1).

Table. 1: Effect of Quercetin-3-O-β-glucoside treatment on the survival time of tumour bearing mice.

Treatment	MST (in days)	Life span %
Saline (control)	19	-
5FU (20mg/kg ip)	29	152.63%
Quercetin-3-O-β-glucoside (50mg/kg ip)	25*	131.57%

*p<0.001.

The average number of peritoneal exudates cells per normal mouse was found to be 5.3 ±0.7 x10^6. Quercetin-3-O-β-glucoside (50mg/kg) single treatment enhanced peritoneal cells to 6.8 ±0.6x$10^{6.}$ While two consecutive treatment enhanced the number to 7.3±0.7x10^6 (Table-2).

Table. 2: Effect of Quercetin-3-O-β-glucoside (50mg/kg, ip) treatment on enhancement of peritoneal cell count in normal mice.

Experiment	Number of peritoneal cells(1x10^6)/mouse
Control	5.3 ± 0.7
Treated once	6.8 ± 0.6
Treated twice	7.3* ± 0.7

*P<0.01.

Haemotological parameters of (Table-3) tumour bearing mice on day 14 were found to be significantly altered from normal group. The total WBC count, protein and PCV were found to be increase with a reduction of the haemoglobin content of RBC. In a differential count of WBC, the percent of neutrophils increased while the lymphocyte count decreased. At the same time interval, Quercetin-3-O-β-glucoside (50mg/kg/day-ip) treatment could change those altered parameters to near normal.

Table. 3: Effect of Quercetin-3-O-β-glucoside (50mg/kg/day i.p) on haemotological parameters in mice.

	Hb (g%)	RBC Million/mm^3	WBC Cell/mm^3	Protein (g%)	PCV (mm)	Differential Count		
						Lympho cytes	Neutro phil	Mono cytes
Normal mice	13.7±0.5	3.90±0.14	9776±44	9.0±0.6	15.8±0.7	62±2	30±2	2±0
Tumour bearing mice (14 days)	8.7±0.6	2.62±0.06	24808±108	12.8±1.2	27.0±1.4	34±4	64±8	2±0
Treated tumour bearing mice	10.9±0.6	3.08±0.20	13408±160*	10.7±0.4	20.7±0.6*	50±3	47±2	3±0

***P<0.001**

DISCUSSION

The reliable criterion for judging the value of any anticancer drug is the prolongation of lifespan of lifespan of the animal* and reduction of WBC from blood[30]. The above results demonstrated the antitumour effect of Quercetin-3-O-β-glucoside against DAL in swiss albino mice. A significant enhancement of MST was found. The harvested viable cells (Trypan blue method) after Quercetin-3-O-β-glucoside treatment showed morphological changes as revealed by the reduction in size of the cells. To evaluate whether Quercetin-3-O-β-glucoside treatment indirectly inhibited the tumour growth, the effect of Quercetin-3-O-β-glucoside was examined on the peritoneal exudates of normal mice. Normally each mouse contains about 5x10^6 intraperitoneal cells, 50% of which are macrophages. Quercetin-3-O-β-glucoside treatment was found to enhance peritoneal cells counts. When these Quercetin-3-O-β-glucoside treated animals underwent i.p. inoculation with DAL cells tumour cell growth was found to be

inhibited. These results demonstrated the indirect effect of Quercetin-3-O-β-glucoside on DAL cells, probably mediated through some cytokine product inside the peritoneal cavity produced by Quercetin-3-O-β-glucoside treatment.

Analysis of haemotological parameters showed minimum toxic effects in the mice which were treated with Quercetin-3-O-β-glucoside. After 14 days of transplantation, Quercetin-3-O-β-glucoside treated groups were able to reverse the changes in haemotological parameters consequent to tumour inoculation.

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