Flavonoids are found to be effective biologically active agents. The various pharmacological effects shown by flavonoids include anti-inflammatory, anti-ulcer, anti-allergic, anti-bacterial, anti-fungal, anti-carcinogenic, anti-hepatotoxic, anti-honnonal, anti-oxidant, anti-proliferative, anti-thrombotic and anti-viral activities\(^1\).

The involvement of oxygen free radicals in the cardioprotective effect of rutin, a naturally occurring flavonoid, has been reported by Kanwaljit Chopra and Manjeet Singil\(^2\). Two flavonoids, viz., 5,7,3'-trihydroxy-3,6,4'-trimetlioxyl flavone (centaureidin) and 5,3'-dihydroxy-4'-methoxy-7-carbomethoxy flavonol isolated from *Tanacetum micwphylhum* have shown significant anti-inflammatory activity. The anti-inflammatory and anti-ulcer activities of kaempferol, isolated from *Rhammis procumbesis*, have been reported by Goel et al. Chrysin, a flavonoid is reported to show significant anti-convulsant activity.\(^5\)

Apigenin, luteolin, chrysoeriol, their 7-glucosides and their 7-glucuronides, have already been shown to possess anti-inflammatory activities\(^6\). Isovitexin-6"-0-glucoside, a new flavone-C-glycoside and other flavonoids like quercetin, isovitexin, lutcoim-7-0-(M)-glucoside isolated from
*Gentiana arisanemsis* showed both anti-platelet and vasorelaxing properties\(^7\). Five prenylated flavanones naniely, 6-(1,1-di dimethylallyl)naringenin, 6-(1,1-di methylallyl)eriodictyol, 3’-methoxy-6- (1,1-dimethylallyl)eriodictyol, 6, 8-di prenyleriodictyol and hiravanone, all isolated from the leaves of *Monotes engleri* exhibited cytotoxic activity against human cancer eeli lines\(^8\).

Kaemperol-7-O-a-L-rhamnoside, kaemperol-3, 7-di-O-a-L-rhamnoside and apigenin-7-O-a-D-glucoside, isolated from the leaves of *Hibiscus cannabinus* exhibited anti-bacterial activity\(^9\) The flavonoid constituents of the 70% ethanolic extract of *Anthriscus sylvestris* comprising of quercetin, apigenin and rutin showed strong anti-oxidant properties\(^10\). The methyl and acetyl derivatives of morin, quercetin, naringenin and rutin isolated from joya showed anti-cholesteremic effect in rats\(^9\). The novel prenyl flavones isolated from the leaves of *Epimedium sagillaluin* namely, yinyanghuo A and yinyanghuo B, showed significant anti-platelet activity induced by arachidontc acid\(^12\). The prenylated isoflavanones from *Erythrina erioiricha* are reported to possess anti-microbial properties\(^1\).

The flavonoids of *Polycarporn succulcnlwn* have been reported to possess analgesic, anti-pyretic, anri-inflammatory, diuretic and loeal anaesthetic properties\(^11\). Various substituted flavanones isolated from *Leguminaceae* plants showed anti-bacterial activity\(^15\). The flavonoid constituents of *PhyUantims* species showed analgesic activity in mice.
The flavones and the flavone glycosides of *Echinops echinatus* have proved to be effective anti-fungal agents\(^\text{17}\). Anti-fungal activity has been observed in the methylated flavanones of *Helichrysum nitidum*. The isoflavanones isolated from the ethanol extracts of the roots of *Desmodium canum* have shown significant anti-microbial activities\(^\text{19}\). A number of flavonoids have been reported to possess anti-bacterial activity against the skin bacterium, *Staphylococcus epidermidis*\(^\text{20}\).

The complex flavanones from *Silybum marianum*, showed anti-hepatotoxic properties\(^\text{21}\). Gryglewski *et al*\(^\text{22}\) have studied the mechanism of the anti-thrombotic action of the flavonoids. Quercetin, morin, rutin, taxifolin, apigenin, catechin, hesperetin and naringenin have been reported to possess anti-viral activity against eleven types of virus\(^\text{23}\). Quercetin has been reported to be capable of inhibiting the stimulated secretion of rat pituitary growth hormone\(^\text{24}\). Kimura *et al*\(^\text{23}\) reported the presence of 5, 7, 2', 5'-tetrahydroxy-6, 8-dimethoxyflavone in the roots of *Scutellaria baicalensis* which inhibited the lipid peroxide formation.

Present work

A review of literature on the pharmacological studies of flavonoids showed that though a large number of flavonoids show a number of pharmacological activities, reports of the pharmacological studies of naturally occurring isoflavones, in particular, are only a few. The occurrence of
Biochanin-A in large quantities in the flowers of *D. sissoides* coupled with the fact that not many isoflavonoids have been screened for their pharmacological activities, encouraged us to carry out the pharmacological screening of Biochanin-A. In this chapter, a few pharmacological effects such as the anti-inflammatory, anti-tumour, hypotensive, locomotor and bioassay studies like larvicidal and anti-bacterial activities of biochanin-A were carried out and reported.

**Isolation of Biochanin-A from the flowers of *Dalbergia sissoides***

*Dalbergia sissoides* (Grah) belonging to the *Leguminosae* family was identified by Prof. Sriganesan, Professor of Botany (retd.), Madura college, Madurai and the flowers (750 g) were collected from the Alagar Hills of Madurai district of South India. The air-dried flowers weresuccessively extracted using hot solvents such as petroleum ether, benzene and alcohol for 36 h (6 x 6 h). The benzene extract, on concentration, gave a crude solid of 4.4 g. This was subjected to column chromatography over a silica gel column. The elution of the column was carried out with solvents in the order of a) benzene, b) benzene-chloroform mixtures with increasing amounts of chloroform, c) chloroform, d) chloroform-alcohol mixtures with increasing amounts of alcohol and e) alcohol.

The fractions eluted with benzene: chloroform (9:1, v/v) yielded a pale yellow solid (250 mg). It showed a melting point of 214-215°C and it
appeared homogenus on TLC. The compound gave a dark blue colour with neutial fenic chloride and it showed a pink color with sodium-amalgam and HCl (Wolfroni test\textsuperscript{26} for isoflavones). It did not answer the Shinoda test\textsuperscript{27} for flavonoids, Durham test\textsuperscript{28} for isoflavanones and Molisch test\textsuperscript{29} for glycosides. The compound showed the following spectral data.

**UV spectral data of the compound**

\[
KTM \\
\text{MeOH} & 263 \text{ nm} + \text{NaOMe} & 275 \text{ uni} \\
+ \text{AlCl}_3 & 266 \text{ nm} + \text{AlCl}_3 + \text{HCl} & 270 \text{ nm} \\
+ \text{NaOAc} & 270 \text{ nm} + \text{NaOAc} + \text{H}_3\text{BO}_3 & 263 \text{ nm}
\]

**SR spectral data of the compound**

\[(v, \text{KBr}) \ 3258, 1624, 1515, 1175, 1044, 1008, 896, 830, 701, 653, 617, 575, 530, 503 \text{ and 471 cm}^\text{-1}.

**PMR spectral data of acetate of the compound**

\[(8, \text{CDCl}_3 \text{-} 200 \text{ MHz}) \ 2.30 \ (3\text{H, s, OCOCH}_3); 2.40 \ (3\text{H, s, OCOCH}_3); 6.85 \ (1\text{H, d, J=}3\text{Hz,H-6}); 6.45 \ (1\text{H, d J=}3\text{Hz, H-8}); 6.95 \ (2\text{H, d (J=}9\text{Hz), H-3'} \text{ and H-5'}); 7.20 \ (1\text{H, d (J=}9\text{Hz), H-2'} \text{ and H-6'}) \text{ and 7.85 (IH, s, H-2) ppm}

The UV data indicated the compound to be a 5,7-dihydroxyisoflavone.
The diacetate formed by acetylation of the compound with acetic anhydride and pyridine had a melting point of m.p. 188-189°C. From the melting point of the parent compound and its acetate and from the UV and PMR spectral data, the compound was identified as Biochanin-A (5,7-dihydroxy-4'-methoxy isoflavone) already reported from a number of Dalbergia species. The compound was further confirmed to be Biochanin-A by co-TLC and mixed m.p of it with an authentic sample of Biochanin-A.

The pharmacological and the bioassay studies carried out with Biochanin-A are:

1) Anti-inflammatory activity
2) Anti-tumor activity
3) Hypotensive activity
4) Locomotor activity
5) Anti-larval activity
6) Anti-bacterial activity

ANTI-INFLAMMATORY ACTIVITY OF BIOCHANIN-A

The anti-inflammatory activity of Biochanin-A against carrageenan-induced acute paw oedema was tested in rats and was compared with a standard drug ketorolactromethamine.

Experimental Methodology

The principle, equipment and the methodology followed in this experiment were similar to that discussed in chapter IV. Biochanin-A at a close of 20 mg/kg was used in this study. Carrageenan (1% w/v solution was
prepared and 0.1ml was injected under the plantar region of the left paw) was used as the inflammagen. The standard drug ketorolactomethamine was used at a dose of 10 mg/kg. The group administered with vehicle alone served as the control group. The paw volumes of the control, Biochanin-A treated group and the standard groups were noted at 30, 60, 120, 180, 240, 300 and 360 minutes after carrageenan challenge. The experimental results were analysed using Dunnett's t-test and the results obtained at a time duration of three hours is given in Table- 24

**TABLE – 24**

**Effect of Biochanin-A on carrageenan induced rat paw oedema**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Paw oedema volume after 3h (mean ± SEM)</th>
<th>% inhibition of paw oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5 ml/kg (distilled H₂O)</td>
<td>0.318 ± 0.04</td>
<td>*</td>
</tr>
<tr>
<td>Ketonolactromethamine</td>
<td>10</td>
<td>0.172 ± 0.02 *</td>
<td>45.92</td>
</tr>
<tr>
<td>Biochanin-A</td>
<td>20</td>
<td>0.204 ± 0.04 *</td>
<td>35.85</td>
</tr>
</tbody>
</table>

*P < 0.01

**Results and Discussion**

The standard anti-inflammatory drug ketorolactromethamine showed significant reduction in the paw oedema volumes when compared to the untreated control group. This indicated that the model employed for the present study was appropriate and the data, thus obtained could be tested for the level of significance for the anti-inflammatory activity of Biochanin-A.
A maximum anti-inflammatory activity due to Biochanin-A was observed at a time interval of three hours after drug administration.

The statistical analysis of the data showed that the test drug, Biochanin-A, exhibited a significant anti-inflammatory activity, when compared to the untreated control group. It can be seen from the Table-24, that the test drug, Biochanin-A, showed significant percentage inhibition of paw oedema, which was comparable with the standard drug, ketorolactromethamine. The significant anti-inflammatory activity of Biochanin-A may be attributed to its histamine, kinin and prostaglandin inhibitory activity.

ANTI-TUMOR ACTIVITY OF BIOCHANIN-A

Flavonoids, off late, have developed immense reputation as excellent anti-oxidants and enough reports have been found in literature to prove the anti-tumor and anti-cancer properties of flavonoids". In the present study the anti-tumor activity of biochanin-A, isolated from Dalbergia sissoides flowers is evaluated against Dalton's ascitic lymphoma (DAL) eelis inoculated in mice.

Experimental Methodology"

Biochanin-A was dissolved in phospliate buffer with a pH of 7.4. Swiss albino mice (20-24 g), housed in standard microlon boxes were used and were given standard laboratory diet and waler ad iibiiium.
DaltoiT's ascitic lymphoma (DAL) cells obtained through the courtesy of Cancer Research Institute, Adyar, Chennai, were used for the study. DAL cells were maintained by weekly intraperitoneal (i.p) inoculation of $10^6$ cells/mouse.

Animals were inoculated with $2-3 \times 10^5$ cells/mouse on day 0, and treatment with Biochanin-A started 24 h after inoculation, at a dose of 10 mg / kg i.p (group A). The control group (group-B) was treated with the same volume of 0.9% sodium chloride. All treatments were continued for nine days. Median survival times (MST) for each group, containing 10 mice were noted. The anti-tumor efficacy of Biochanin-A was compared with that of 5-fluorouracil (5 FU) (20 mg/ kg/ day i.p. for 9 days). MST was noted with reference to the control.

Survival times of the treated groups (T), were compared with those of control groups (C) by using the following formula:

$$\text{Increase of life} = \frac{(T/C)}{\text{MST of treated group} \times 100} \times \frac{\text{MST of control group}}{\text{MST of treated group}}$$

The results are tabulated in Table-25. The effect of Biochanin-A on the survival of tumor bearing mice showed MST for the control group to be 23 days, while it was 31 days and 40 days for the groups treated with Biochanin-A (10 mg/ kg/ day i.p.) and 5FU (20 mg/ kg/ day i.p.) respectively. Studies of in vivo tumor cell growth inhibition with Biochanin-A were carried out under similar experimental conditions as stated above, using the dose of 10 mg/kg/day for 6 days.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose of the drugs (mg/kg)</th>
<th>MST (in days)</th>
<th>Increase in life span T/C %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU</td>
<td>20</td>
<td>40</td>
<td>173.90</td>
</tr>
<tr>
<td>Biochanin-A</td>
<td>10</td>
<td>31</td>
<td>134.70 *</td>
</tr>
<tr>
<td>Saline</td>
<td>Same vol. 0.9% NaCl sol</td>
<td>23</td>
<td>100</td>
</tr>
</tbody>
</table>

* P < 0.01

Number of animals used: 10 in each group
Days of drug treatment: nine

Animals were sacrificed on day 7 after inoculation and tumor cells were collected by repeated intraperitoneal wash with 0.9% NaCl. Viable tumor cell counts (trypan blue test) were made using a haemocytometer. Total number of viable cells per animal of the treated group was compared with those of the control group.

On day 7 after inoculation, the i.p., DAL cell count of the treated group was $2.48 \pm 0.34 \times 10^8$ cell/mouse. This value was significant (p < 0.01) when compared to that of control. The sizes of the DAL cells harvested from treated mice were appreciably smaller with respect to the control cells.
In another experiment three groups of normal mice (n = 5) were used for the study the effect of Biochanin-A on peritoneal cell count. One group was treated with 10 mg/kg i.p for one day while the other group received the same treatment for two consecutive days. The untreated third group was used as control. Peritoneal exudate cells were counted 24 h after treatment for each of the treated groups and compared with the untreated group. The observations are tabulated in Table-26

TABLE-26 Effect of Biochanin-A (10 mg/kg) treatment on the Enhancement of Peritoneal cells in normal mice

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of peritoneal cells x 10^6/ mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.1 ± 0.8 x 10^6</td>
</tr>
<tr>
<td>Treated once</td>
<td>7.7 ± 0.6 x 10^6</td>
</tr>
<tr>
<td>Treated twice on two consecutive days</td>
<td>8.6* ± 0.5 x 10^6</td>
</tr>
</tbody>
</table>

*P<0.01; Number of animals used : 5 in each group; Values expressed as mean ± SEM

The average number of peritoneal exudate cells per normal mouse was found to be 6.1 ± 0.8 x 10^6. Biochanin-A (10 mg/kg) treatment increased the number of the peritoneal cells as shown in Table-26. Single treatment enhanced peritoneal cells to 7.7 ± 0.6 x 10^6 while two consecutive treatments enhanced the number to 8.6 ± 0.5 x 10^6.
In order to detect the influence of Biochanin-A on the haematological status of DAI bearing mice, comparison was made amongst three groups (n=5) of mice on the 14th day after inoculation. The three groups were:

1) tumor bearing mice, 2) tumor bearing mice treated with Biochanin-A (10 mg/Kg/day i.p. for 9 days) and 3) control group. Blood was drawn from each mouse in the conventional way and the white blood cell count, red blood cell count, haemoglobin, protein and packed cellular volume (PCV) were determined\(^{39,40}\). All the results were analyzed by analysis of variance\(^{41}\) and the results are tabulated in Table -27

**TABLE - 27 Effect of Biochanin-A (10 mg/kg) on the Hematological parameters**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb</th>
<th>Total RBC cells x10^{10}/ml</th>
<th>Total WBC cells x10^{6}/ml</th>
<th>Protein g%</th>
<th>PCV mm</th>
<th>Differential count %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lymphocyte</td>
</tr>
<tr>
<td>Normal mice</td>
<td>15.2 ±0.8</td>
<td>1.45±0.2</td>
<td>7.8±0.6</td>
<td>8.7±0.7</td>
<td>17±0.6</td>
<td>68±3</td>
</tr>
<tr>
<td>Tumor bearing mice</td>
<td>12.6 ±0.4</td>
<td>1.26±0.4</td>
<td>14.2±0.7</td>
<td>1.26±0.8</td>
<td>24±0.7</td>
<td>30±2</td>
</tr>
<tr>
<td>(14 days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated tumor</td>
<td>13.1 ±0.7</td>
<td>1.32±0.15</td>
<td>10.7*±0.4</td>
<td>10.4±0.8</td>
<td>20±0.8</td>
<td>57*±3</td>
</tr>
<tr>
<td>bearing mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.01; Number of animals : 5 in each group;
Days of drug treatment : 9; values expressed as mean ± SEM
Haematological parameters of tumor bearing mice on day 14 were found to be significantly alter from the normal group. The total WBC counts, protein and PCV were found to increase with Üie reduction in 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 Biochanin-A (10 mg/ kg/ day i.p.) treatment could change those altered parameters to near nonnal.

Results and discussion

The reliable criterion for judging the value of any anti-cancer drug is the prolongation of lifespan of the ämmal and disappearance of WBC from blood ”. The above results demonstrated the anti-tumour effect of biochanin-A against DAL in swiss albino mice. A significant enhancement of MST was found. Our observation of inhibition of in vivo tuinor eeli growth after Biochanin-A treatment appeared to correlate with the finding of enhancement of survival time by Biochanin-A with respect to the control. The harvested viable eelis after Biochanin-A treatment showed morphological changes as revealed by a reduction in the size of the eelis. Biochanin-A treatment was found to enhance peritoneal eeli counts. Analysis of the hematologica! parameters showed minimum toxic effects in mice on Biochanin-A treatment. After 14 days of transplantation, Biochanin-A treated groups were able to reverse the changes in the hematological parameters as a consequence of tumor inoculation.
HYPOTENSIVE ACTIVITY OF BIOCHANIN-A

In an attempt to realise the potency of Biochanin-A as a further pharmacologically active natural product, the effect of Biochanin-A on the cardio-vascular system was studied by using anaesthetized dog preparation.¹⁴

The heart, blood vessels and the lungs (bronchial smooth muscles) are under the control of the autonomic nervous system. Both sympathetic and parasympathetic systems have nervous supply to the heart. Parasympathetic innervation is through the vagus whereas the sympathetic nerve supply comes from the fibers arising from satellite or inferior cervical ganglion. The nervous supply to the blood vessels is principally from the sympathetic system. In general sympathetic stimulation by administration of adrenaline and noradrenaline increases cardiac output which increases the blood pressure. On the other hand, parasympathetic stimulation by administration of acetylcholine decreases the blood pressure. Drugs that increase the blood pressure are called the pressor agents while those, which decrease, are called the depressor agents.¹⁴

Experimental Methodology

Pentobarbitone sodium at a dose of 35mg/kg i.p. (maintenance dose: 3-4 mg/kg) was used as anaesthetic in the study. The drugs used to study the hypotensive activity included adrenaline (dose: 5 μg/kg), acetylcholine
(dose 5 ug/kg), histamine (dose-5 ug/kg), Biochanin-A (Ds) (doses-5 fig, 10 p.g, 20 ug and 40 (ig/kg) body weight and chlorpheniramine maleate (at a dose of 1 mg/kg).

Dog weighing between 10-15 kg was anaesthetized with 35-40 mg/kg of pentobarbitone sodium given intraperitoneally. The femoral vein was cannulated (by venous cannula) and connected to a burette containing normal saline. The saline was allowed to flow slowly while the drugs to be studied were injected through the rubber tubing connecting the cannula to the burette containing saline. Carotid artery was cannulated (by arterial cannula) and connected to a mercury manometer through a tube filled with anti-coagulant fluid. The pressure in the manometer was increased. The base line mean arterial pressure was recorded on the smoked paper bound on a kymograph when the mercury level in the other arm of the manometer showed constant level.

Initially the "tetrad responses" were recorded on the kymograph. The different tests, which constitute the normal response brackets were:

1) occluding both carotid arteries for 45 seconds and recording the effect on blood pressure (BP).

2) electrical stimulation of the peripheral vagus nerve (20 V electric shocks/sec for 3 sec and recording the effect on BP.

3) injecting adrenaline (5 ug/kg) through femoral venous cannula and recording the effects in the B.P till it returned to the normal base line.
4) injecting acetyicholine (5 M-g/kg) through femoral venous cannuia and recording the effects in the B.P till it retumed to the normal base line.

The tetrad responses are recorded to establish that the test animal and the test procedure carried out are suitable for studying the cardiovascular activity of the testdrug.

Histamine (dose - 5 j_ig/kg) vwas injected tlirough femoral venous cannuia and recorded the effects in the B.P till it retumed to the normal base line. Biochanin-A, the test drug (labelled Dsf in the kymograph) vwas injected tlirough femoral venous cannuia in three doses (doses - 5 yig, 10 ug, and 20 j.ig/kg body weight) and recorded the effects in the B.P till it retumed to the normal base line. The tetrad responses were recorded once again to ascertaiii the stability of the test procedure. Chlorpheniramine maleate (dose - 1 mg/kg), au anti-histaminic agent was injected tlirough femoral venous cannuia followed by two doses (doses - 20 ug and 40 jig/ kg body weight) of biochanin-A (Dsf) and recorded the effects in the blood pressure till it retumed to the normal base line. The results obtained are shown in the kymograph as shown in Fig 37

Kestilts and Discussion

The initial tetrad responses observed showed that. the test animal and the procedure employed are suitable for the studies to assess the cardiovascular activity of the test drug in the present sttidy.
The test drug, Biochanin-A produced significant reduction in the blood pressure, which is dose-dependant. It is comparable with 5 pg/kg of histamine. Both Biochanin-A and histamine- induced hypotension is conveniently blocked by the prior administration of anti-histaminic agent namely, chlorpheniramine maleate (1 mg/kg). It has been seen that Biochanin-A did not produce any significant effect on guinea pig ileum and isolated heart preparation as histamine does. From all these experiments, it is clear that Biochanin-A acts in a similar mechanism to that of histamine in cardiovascular system but for the difference in activity on isolated heart and guinea pig ileum. Biochanin-A may be acting as a histamine liberator in intact animals but the anti-inflammatory effect of the drug contradicts the same. Perhaps the anti-inflammatory activity of the test drug Biochanin-A may be due to the other non-histaminic pathways.

LOCOMOTOR ACTIVITY OF BIOCHANIN-A

In the present study of investigating further possible pharmacological effects of biochanin-A, an attempt was made to explore the effect of Biochanin-A on the central nervous system and hence the locomotor activity of biochanin-A was evaluated using a standard method by employing actophotometer.

Experimental Methodology

The experiment was carried out according to the procedure suggested by Kui kärni.
The test drug Biochanin-A at doses of 20 mg/kg and 10 mg/kg, was evaluated for its locomotor activity and compared with standard drug Diazepam. Diazepam (at a dose of 1.5 mg/kg i.p.) was given as an injection (1 ml/100 g body weight of the rat) from a stock solution containing 0.15 mg/ml of the drug. The untreated group, administered with vehicle alone, served as the control. The changes in the locomotor activity in all the groups were noted before and after the drug administration. The observations were tabulated as shown in Table-28. The values in the table correspond to the mean values for each group. Statistical analysis (Paired t-test) was done in order to ascertain the level of significance of the test drug Biochanin-A. The statistical data obtained were tabulated as shown in Table-29.

**TABLE -28 Experimental data of locomotor activity of Biochanin-A**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Dose of drug (mg/kg)</th>
<th>Locomotor activity (mean values)</th>
<th>% reduction in locomotor activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before drug injection</td>
<td>After drug injection</td>
</tr>
<tr>
<td>Biochanin-A</td>
<td>20</td>
<td>79.83</td>
<td>33.83</td>
</tr>
<tr>
<td>Biochanin-A</td>
<td>10</td>
<td>126.33</td>
<td>52</td>
</tr>
<tr>
<td>Std. drug</td>
<td>1.5</td>
<td>103.5</td>
<td>42.3</td>
</tr>
<tr>
<td>(diazepam)</td>
<td>Control</td>
<td>155</td>
<td>151.8</td>
</tr>
</tbody>
</table>
TABLE -29 Statistical data of locomotor activity of Biochanin-A

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose of the drugs (mg/kg)</th>
<th>% reduction in locomotor activity Mean ± SEM</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>2.55 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>Std(diazepam)</td>
<td>1.5</td>
<td>58.78 ± 4.15</td>
<td>4.58 **</td>
</tr>
<tr>
<td>Biochanin-A</td>
<td>20</td>
<td>59.77 ± 7.3</td>
<td>7.87 ***</td>
</tr>
<tr>
<td>Biochanin-A</td>
<td>10</td>
<td>58.14 ± 3.42</td>
<td>5.66 **</td>
</tr>
</tbody>
</table>

Paired t-test: *P< 0.05  **P < 0.01  *** P < 0.001

Results and discussion

It is evident from the Tables-28 and 29 that the test drug, Biochanin-A, showed significant reduction in the locomotor activity, when compared to the untreated control group. From the tables, it is also clear that Biochanin-A showed very significant reduction (P < 0.001) at a dose of 20 mg/kg. It is also observed that the percentage reduction in the locomotor activity at both the doses of 10 mg/kg and 20 mg/kg were comparable to that of the standard drug, diazepam. Further, it was found that Biochanin-A, at a dose of 20 mg/kg showed 59.77% reduction, which was higher than that of the standard drug (58.78%).
ANTI-LARVAL ACTIVITY OF BIOCHANIN-A

Stagnated water is a major environmental hazard as it poses a serious problem by supporting the breeding of mosquitoes, which are world's number one pests. A number of natural and synthetic pesticides have been identified around the world. A majority of the synthetic insecticides cause harmful effects on humans and animals and also cause environmental pollution. In the search for safe and congenial alternatives, plant derivatives have been extensively examined and it has been found that the secondary metabolites of these plants affect survival, growth and metamorphosis of the insects.

Flavonoids, which constitute a major portion of the secondary metabolites in plants, have been found to affect the physiological systems in higher animals, insects, microbes etc and hence was considered suitable for this study. In the present study the isoflavone, Biochanin-A isolated from the flowers of Dalbergia sissouies, as described earlier, was assessed for its larvicidal activity on the fourth instar larvae of Culex quinquefasciatus (Say).

In normal larvicidal studies, the solubility of water insoluble test compound in water (water solubility highly desirable) is enhanced by dissolving the compound in acetone before mixing with water as acetone by itself has very little effect on the pests. But this may result in evaporation of acetone leaving a deposit of water insoluble compound at the bottom of the water body (both eluring laboratory experiments and Hekl studies). This may
result in improper results, which may be misleading. Hence in the present study a novel technique of converting the water insoluble isoflavone Biochanin-A into its water soluble sodium salt was employed. The effect of alkali used for preparation of the sodium salt of Biochanin-A as the larvicidal agent was studied separately by taking sodium hydroxide solution as control.

Experimental Methodology

Biochanin-A was prepared by dissolving 115 mg in 7 ml of 0.2N aqueous sodium hydroxide and made up to 100 ml to obtain a 1150 ppm stock solution of the sodium salt of Biochanin-A. Egg rafts of *C. quinquefasciatus* were obtained from the Centre for Research in Medical Entomology (ICMR), Madurai, South India, from which the laboratory colonies of the vector used in this study were developed and maintained at 28 ± 2°C, 75-85% RH and under a 14 L: 10 D photoperiod cycle.

From the stock solution, various concentrations of the sodium salt of Biochanin-A (50, 100, 150, 200, 250 ppm) were prepared each in 150 ml of distilled water. Three replicates of each concentration were maintained throughout the experimental period. Twenty larvae of the fourth instar of *C. quinquefasciatus* were introduced into each of the test solutions of different concentrations. The larvae were fed with a diet of yeast and dog biscuits (3:1).

In order to find out the effect of alkalinity on the mortality of the larvae, solutions of NaOH of different concentrations (64, 128, 192, 256 and 320 ppm
as Na’) were prepared, each in 150 ml of distilled water and the experiments were repeated as before. The mortality of larvae in these solutions was noted up to 24 h. The effect of NaOH on the mortality of the fourth instar larvae of *Gquinquifasciatus* was observed up to 48 h also to find out whether NaOH has some effect on the test organism or not. The results obtained are tabulated in Table-31.

The Statistical evaluation of the data was carried out by the use of Prohil Analysis. The results were compared with the control (121.7 ppm Na) equivalent to the highest concentration of sodium in the test solutions. The experimental data, which includes the percentage mortality of the larvae and the statistical analysis carried out, are tabulated in Table-30.

Results and Discussion

The 24 h mortality, LC$_{50}$, LG, and chi-square values, regression equation and the Model limits for LC$_W$ and LU, are shown in Table-30. The chi-square value showed high significance ($P<0.01$). Hence the experimental data can be interpreted to find the LC$_{50}$ and IC$_{50}$ of the compound. The regression equation was employed to calculate $U'_{wa}$ and IC$_{50}$, and the values were found to be 306.238 ppm and 18B.926.
Table-30  Effect of the sodium salt of Biochanin-A against fourth instar larvae of *Culex quinquefasciatus*

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Larval mortality % (24h)</th>
<th>Log LC₅₀</th>
<th>LC₅₀</th>
<th>Chi Square</th>
<th>Regression Equation</th>
<th>95% fiducial effect LC₅₀ (LC₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>64</td>
<td>2.486</td>
<td>306.238</td>
<td>39.995</td>
<td>Y = 0.9682 +1.622 X</td>
<td>1.118956 UL 695.29</td>
</tr>
<tr>
<td>200</td>
<td>24.4</td>
<td>3.276</td>
<td>1889.926</td>
<td>P&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>22.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>21.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>20.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table-31 Effect of alkalinity on the fourth instar larvae of *Culex quinquefasciatus* (control)

<table>
<thead>
<tr>
<th>Conc. Of NaOH as Na⁺ (ppm)</th>
<th>Mortality after 24 h (%)</th>
<th>Mortality after 48 h (%)</th>
<th>Pupal development (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>128</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>192</td>
<td>0</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>256</td>
<td>0</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>320</td>
<td>5</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

It is observed that the sodium salt of Biochanin-A, at a concentration of 250 ppm, has significant anti-larval activity under the test conditions.
A decrease in the larvicidal activity was observed with the reduction in the concentration of the test drug.

The alkalinity due to NaOH (as Na') was found to have no mortal effect upto 256ppm upon the test organisms for 24 h. However, further observations made upto 48h showed that there was a mortality of 5%, 15% and 40% at 192, 256 and 320 ppm respectively, as shown in the Table-31. As the concentration of NaOH solution used in our study was only 50% of the test solution, the observations show that NaOH, by itself, had no effect upon the larvae at these low concentrations.

ANTI-BACTERIAL ACTIVITY OF BIOCHANIN-A

The anti-bacterial activity of Biochanin-A was carried out on the basis of the procedure suggested by Leven\textsuperscript{56} as explained in chapter IV. The method is based on the diffusion of anti-bacterial principle from reservoir hole to the surrounding inoculated agar medium such that the growth of the microorganism is inhibited as circular zone around the hole. The microorganisms used for the experiments, the culture medium used and the procedures employed in this experiment were similar to that explained in chapter IV. The results are tabulated as shown in Table-32.
### TABLE-32 Anti-bacterial activity of Biochanin-A

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Drug</th>
<th>Concentration (mg/ml)</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S.A</td>
</tr>
<tr>
<td>1</td>
<td>Biochanin-A</td>
<td>6.25</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.50</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Streptomycin (standard)</td>
<td>100</td>
<td>20</td>
</tr>
</tbody>
</table>

S.A - *Staphylococcus aureus*,  
B.S - *Bacillus subtilis*,  
E.C - *Escherichia coli*,  
P.A - *Pseudomonas aeruginosa*

**Results and Discussion**

It can be seen clearly from the above table that the test drug, Biochanin-A, has not shown any anti-bacterial activity in the present model employed against any of the four microorganisms, chosen for the present study.
REFERENCES


33. Banerjee A., Murti V. V. S., Seshadri T. R and Thakur R. S., Indian J
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