Chapter – 6

DISCUSSION OF RESULTS
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In the process of making new drugs available for the treatment of various ailments, ethanopharmacology plays an important role. In spite of the development of latest techniques in the drug discovery by synthetic means, the search for new natural sources of drugs is continuing and hence the importance of ethanopharmacology is enormous. Of all the new chemical entities launched into the market in the last two decades, one third is of natural origin.

The high inorganic content represented by quantity of ash due to the presence of Na$^+$ and Ca$^+$ salts indicates its safety$^{247}$. Macronutrients and heavy metals were found to be within the limits of pharmacopoeia$^{248-250}$.

Phytochemical studies of FCE showed the presence of carbohydrates, flavonoids, proteins, steroids and tannins. Alkaloids, flavanoids, triterpenoids, tannins, phenolics, carbohydrates and proteins were found to be present in GAE$^{251-253}$.

Acute toxicity studies of FCE and GAE were conducted as per OECD guidelines 425. The FCE and GAE did not show any sign and symptoms of toxicity or mortality up to 5000 mg/kg body weight on oral administration, thus these extracts could be considered as category 5. Body weight before and after administration were noted and any variations in skin, fleece, eyes, mucous membranes, breathing, autonomic, central nervous system, interactive pattern,
signal of tremors, salivation, diarrhea, fatigue, sleep and unconsciousness were observed.

Compound 1, \( \text{FCE}_1 \), is a white powder with MP 136-140\(^\circ\)C and MW 414.71 at 25\(^\circ\)C.

**IR:**

\[
\begin{align*}
\text{OH} & : 3400, 1056 \text{ cm}^{-1} \\
\text{Hydrogen skeleton} & : 2960, 2870, 1706, 1450 \text{ cm}^{-1} \\
\text{-C=C=} & : 965, 802 \text{ cm}^{-1}
\end{align*}
\]

**NMR:**

\( ^1\text{H} \) NMR (500 MHz, CDCl\(_3\)): \( \delta \) (ppm) = 0.74 (1H, s, H-18), 0.87 (\( ^1\text{H}, d, J = 6.9 \text{ Hz}, \text{H-27} \)), 0.88 (\( ^1\text{H}, d, J = 6.9 \text{ Hz}, \text{H-26} \)), 0.89 (\( ^1\text{H}, t, J = 7.4 \text{ Hz}, \text{H-29} \)), 0.93 (\( ^1\text{H}, d, J = 6.5 \text{ Hz}, \text{H-21} \)), 0.97 (\( ^1\text{H}, m, \text{H-24} \)), 0.98 (\( ^1\text{H}, m, \text{H-9} \)), 1.04 (\( ^1\text{H}, m, \text{H-14} \)), 1.06 (\( ^1\text{H}, s, \text{H-19} \)), 1.07 (\( ^1\text{H}, m, \text{H-22b} \)), 1.11 (\( ^1\text{H}, tm, J = 11.2 \text{ Hz}, \text{H-15b} \)), 1.13 (\( ^1\text{H}, m, \text{H-1b} \)), 1.16 (\( ^1\text{H}, t, J = 10.0 \text{ Hz}, \text{H-17} \)), 1.21 (\( ^1\text{H}, m, \text{H-23} \)), 1.21 (\( ^1\text{H}, m, \text{H-12b} \)), 1.30 (\( ^1\text{H}, m, \text{H-16b} \)), 1.31 (\( ^1\text{H}, m, \text{H-28} \)), 1.36 (\( ^1\text{H}, m, \text{H-22a} \)), 1.40 (\( ^1\text{H}, m, \text{H-20} \)), 1.50 (\( ^1\text{H}, qd, J = 10.8; 4.6 \text{ Hz}, \text{H-11b} \)), 1.50 (\( ^1\text{H}, m, \text{H-7} \)), 1.55 (\( ^1\text{H}, m, \text{H-11a} \)), 1.56 (\( ^1\text{H}, m, \text{H-2b} \)), 1.63 (\( ^1\text{H}, m, \text{H-15a} \)), 1.71 (\( ^1\text{H}, m, \text{H-25} \)), 1.88 (\( ^1\text{H}, m, \text{H-2a} \)), 1.89 (\( ^1\text{H}, m, \text{H-16a} \)), 1.90 (\( ^1\text{H}, m, \text{H-1a} \)), 2.03 (1H, \( td, J = 12.1; 2.4 \text{ Hz}, \text{H-8} \)), 2.06 (\( ^1\text{H}, dt, J = 12.8; 3.6 \text{ Hz}, \text{H-12a} \)), 2.30 (\( ^1\text{H}, td, J = 11.0; 2.0 \text{ Hz}, \text{H-4b} \)), 2.34 (\( ^1\text{H}, ddd, J = 13.0; 5.0; 2.0 \text{ Hz}, \text{H-4a} \)), 3.58 (\( ^1\text{H}, tt, J = 11.3; 5.3 \text{ Hz}, \text{H-3} \)), 5.40 (\( ^1\text{H}, dd, J = 5.2; 2.3 \text{ Hz}, \text{H-6} \)).
The $^1$H NMR spectra of FCE$_1$ showed the presence of six methyl signals that appeared as two methyl singlets at $\delta$ 0.68, and 1.01; three methyl doublets that appeared at $\delta$ 0.81, 0.83, and 0.93; and a methyl triplet at $\delta$ 0.84. The $^1$H NMR spectra of FCE$_1$ also showed one olefinic proton at $\delta$ 5.36. The absence of protons corresponding to the double bond between C-20/C-21 in FCE$_1$ together with the appearance of mass spectral data suggested the presence of a trisubstituted double bond at C-5/C-6 in its structure. The $^1$H NMR spectra of showed a proton corresponding to the proton connected to the C-3 hydroxy group which appeared as a triplet of doublet of doublets at $\delta$ 3.53.

$^{13}$C NMR (125MHz, CDCl$_3$): $\delta$ (ppm) = 11.8 (CH$_3$, C-18), 12.0 (CH$_3$, C-29), 18.8 (CH$_3$, C-21), 19.0 (CH$_3$, C-27), 19.4 (CH$_3$, C-19), 19.8 (CH$_3$, C-26), 21.1 (CH$_2$, C-11), 23.0 (CH$_2$, C-28), 24.3 (CH$_2$, C-15), 26.0 (CH$_2$, C-23), 28.2 (CH$_2$, C-16), 29.1 (CH, C-25), 31.6 (CH$_2$, C-2), 31.8 (CH, C-8), 31.9 (CH$_2$, C-7), 33.9 (CH$_2$, C-22), 36.1 (CH, C-20), 36.5 (C, C-10), 37.2 (CH$_2$, C-1), 39.7 (CH$_2$, C-12), 42.3 (CH$_2$, C-4), 42.3 (C, C-13), 45.8 (CH, C-24), 50.1 (CH, C-9), 56.0 (CH, C-17), 56.7 (CH, C-14), 71.8 (CH, C-3), 121.7 (CH, C-6), 140.7 (C, C-5).

The $^{13}$C NMR together with COSY, HMQC and HMBC showed twenty nine carbon signal including six methyl, eleven methylene, ten methane and three quaternary carbons.

**Mass spectrum**
Mass spectrum (FAB-MS): \( m/z 414 \) (M\(^+\)), \( m/z 396 \) (M-33), 273(M-side chain), 255(M-side chain-42), 213 (231-H\(_2\)O).

All the recorded data such as IR, \(^1\)HNMR, \(^{13}\)CNMR and mass spectrum conclusively prove that the isolated steroidal compound may be 17-(5-Ethyl-6-methylheptan-2-yl)-10, 13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-\(^1\)H-cyclopenta [\( \alpha \)] phenanthren-3-ol i.e. “\( \beta \)-SITOSTEROL” (C\(_{29}\)H\(_{50}\)O).

\[
\begin{align*}
\text{\( \beta \)-sitosterol}
\end{align*}
\]

Compound 2, FCE\(_2\), is a yellow to greenish yellow crystalline powder with MP 316\(^\circ\)C, and MW 302.236

**IR**

\[
\begin{align*}
\text{OH} & : \quad 3500 \text{ cm}^{-1} \\
\text{-C=O} & : \quad 1662 \text{ bcm}^{-1} \\
\text{-C=C=} & : \quad 1614, 1512 \text{ cm}^{-1}
\end{align*}
\]

**NMR:**

\(^1\)H NMR (500 MHz, MeOD): \( \delta \) (ppm) = 6.18 (\(^1\)H, \( d \), \( J = 2.0 \) Hz, H-6), 6.39 (\(^1\)H, \( d \), \( J = 2.0 \) Hz, H-8), 6.88 (\(^1\)H, \( d \), \( J = 8.3 \) Hz, H-5\(^\prime\)), 7.62 (\(^1\)H, \( dd \), \( J = 8.3; 2.1 \) Hz, H-6\(^\prime\)), 7.74 (\(^1\)H, \( d \), \( J = 2.1 \) Hz, H-2\(^\prime\)).
$^{13}$C NMR (125MHz, MeOD): $\delta$ (ppm) = 94.6 (CH, C-8), 99.4 (CH, C-6), 104.7 (C, C-10), 116.1 (CH, C-2’, C-5’), 121.8 (CH, C-6’), 124.3 (C, C-1’), 137.2 (C, C-3), 146.3 (C, C-3’), 148.2 (C, C-2), 150.3 (C, C-4’), 158.4 (C, C-9), 162.6 (C, C-5), 165.7 (C, C-7), 177.5 (C, C-4).

ESI-MS: $m/z$ 300.9 [M-H]-, 602.6 [2M-H]-.

All the recorded data such as IR, $^1$HNMR, $^{13}$CNMR and mass spectrum conclusively prove that the isolated steroidal compound it may be 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4$H$-chromen-4-one i.e. “Quercetin” (C$_{15}$H$_{10}$O$_7$).

![Quercetin](image)

Compound 3, **FCE$_3$**, is a yellow powder with MP 260-265$^\circ$C and MW 284.26

**IR (KBR)**

- $\text{-OH}$ : 3450 cm$^{-1}$
- $\text{-C=O}$ : 1660 cm$^{-1}$
- $\text{-C=C=}$ : 1610, 1505 cm$^{-1}$
- $\text{OCH}_3$ : 2940 cm$^{-1}$

**$^1$H NMR (DMSO-$d_6$)**

$\delta$3.3-3.2 : (S, 2H, -OH)
$\delta$3.8 : (S, 3H, -OCH$_3$)
δ5.7 : (S, 1H, -CH=C=)
δ6.3 : (d, 1H, -H-6)
δ6.8 : (d, 1H, -H-8)
δ6.9 : (d, 1H, -H-6’)
δ7.9 : (d, 1H, -H-5’)

\textbf{\textsuperscript{13}C NMR (DMSO-d\textsubscript{6}):}

<table>
<thead>
<tr>
<th>C</th>
<th>\textsuperscript{13}C NMR (DMSO-d\textsubscript{6})</th>
<th>C</th>
<th>\textsuperscript{13}C NMR (DMSO-d\textsubscript{6})</th>
</tr>
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<tr>
<td>2</td>
<td>164.6</td>
<td>1’</td>
<td>121.6</td>
</tr>
<tr>
<td>3</td>
<td>103.4</td>
<td>2’</td>
<td>128.8</td>
</tr>
<tr>
<td>4</td>
<td>182.3</td>
<td>3’</td>
<td>116.3</td>
</tr>
<tr>
<td>5</td>
<td>157.7</td>
<td>4’</td>
<td>161.8</td>
</tr>
</tbody>
</table>

Mass spectrum (El. MS): m/z 285.13 (M+)

All the recorded data such as IR, \textsuperscript{1}H NMR, \textsuperscript{13}C NMR and mass spectrum conclusively prove that the isolated steroidal compound may be 5,7-dihydroxy-2-(4-methoxyphenyl)chromen-4-one, i.e. “Acacetin” (C\textsubscript{16}H\textsubscript{12}O\textsubscript{5}).

![Acacetin](image)

Compound 4, FCE\textsubscript{4}, is a pale yellow crystalline solid, with M.P.242 °C (468 °F; 515 K) and MW 610.52
\(^1\)H NMR in DMSO \(-d_6\) at 303.13 MHZ (\(\delta_{ppm}\)) 1.00 (d, \(^3\)H, \(J_1=6\)Hz, CH3-Rha), 3.0-3.38 (m, 8H- of sugar moieties), 3.38-3.42 (H-6 Glu, over lapped), 3.71 (dd, \(J_1=9.9\) Hz, \(J_2=4.4\) Hz, H-6 Glu) 4.39 (dd, \(J=1.5\) Hz, H-1 Rha), 5.35 (d, \(J=7.5\) Hz, H-1 Glu), 6.2 (d, \(J=1.8\) Hz, H-6), 6.39 (d, \(J=2.1\) Hz, H-8), 6.85 (d, \(J=9\) Hz, H-5\(^i\)), 7.55 (d, \(J=1.7\) Hz, H-2\(^i\)), 7.56 (dd, \(J_1=2\)Hz, \(J_2=8\)Hz, H-6\(^i\)), 12.61 (s, C5-OH).

\(^{13}\)C NMR in DMSO-\(d_6\) at 75.432 MHz (\(\delta\) ppm): 156.36 (C-2), 133.25 (C-3), 177.25 (C-4), 161.10 (C-5), 98.78 (C-6), 164.00 (C-7), 93.71 (C-8), 156.64 (C-9), 103.99 (C-10), 121.20 (C-1\(^i\)), 115.26 (C-2\(^i\)), 144.65 (C-3\(^i\)), 148.32 (C-4), 116.33 (C-5\(^i\)), 121.63 (C-6\(^i\)), Glucose: 101.26 (C-1\(^{ii}\)), 74.16 (C-2\(^{ii}\)), 76.49 (C-3\(^{ii}\)), 70.07 (C-4\(^{ii}\)), 75.89(C-5\(^{ii}\)), 67.10 (C-6\(^{ii}\)), rhamnose: 100.76 (C-1\(^{iii}\)), 70.46 (C-2\(^{iii}\)), 70.65 (C-3\(^{iii}\)), 71.94 (C-4 \(^{iii}\)), 68.33(C-5 \(^{iii}\)), 17.89 (C-6 \(^{iii}\)).

All the recorded data such as IR, \(^1\)H NMR, \(^{13}\)C NMR and mass spectrum conclusively prove that the isolated compound may be 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[\(\alpha\)-L-rhamnopyranosyl-(1\(\rightarrow\)6)-\(\beta\)-D-glucopyranosyloxy]-4\(H\)-chromen-4-one i.e. Rutin \((C_{27}H_{30}O_{16})\)
Compound 5, \textbf{GAE}_1, a white powder with M.P. 160 to 164°F (320 to 327°F; 433 to 437 K) and MW 355.428. The Molecular ion peak was observed at m/z 355. The Base peak was observed at m/z 354 and the Isotopic peak were observed at m/z 356, m/z 357, and MW 355.428 was found to be (13aS)-2,3,9,10-tetramethoxy-6,8,13,13a-tetrahydro-5H-isoquinolino[2,1-b] isoquinoline i.e. \textbf{Tetrahydropalmatine} (C$_{21}$H$_{25}$NO$_4$)

\begin{center}
\includegraphics[width=0.5\textwidth]{tetrahydropalmatine.png}
\end{center}

\textbf{Tetrahydropalmatine}
Compound 6, **GAE\textsubscript{2}**, is a white powder with M.P. 165-167\textdegree C and MW 412.69

**IR spectrum**

3410 cm\(^{-1}\) - O-H stretching

2955 cm\(^{-1}\) and 2936 cm\(^{-1}\) - aliphatic C-H stretching

1634 cm\(^{-1}\) - C=C absorption peak

Other absorption peaks includes

1461 cm\(^{-1}\) – CH\(_2\)

1383 cm\(^{-1}\) - OH def

1038.7 cm\(^{-1}\) – Cycloalkane.

**NMR**

**\(^1\)H NMR**

<table>
<thead>
<tr>
<th>Position</th>
<th>(\delta) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.51 (tdd, 1H, (J = 4.5, 4.2, 3.8) Hz)</td>
</tr>
<tr>
<td>5</td>
<td>5.31 (t, 1H, (J = 6.1) Hz)</td>
</tr>
<tr>
<td>19</td>
<td>0.91 (d, 3H, (J = 6.2) Hz)</td>
</tr>
<tr>
<td>20</td>
<td>4.98 (m, 1H)</td>
</tr>
<tr>
<td>21</td>
<td>5.14 (m, 1H)</td>
</tr>
<tr>
<td>24</td>
<td>0.83 (t, 3H, (J = 7.1) Hz)</td>
</tr>
<tr>
<td>26</td>
<td>0.82 (d, 3H, (J = 6.6) Hz)</td>
</tr>
<tr>
<td>27</td>
<td>0.80 (d, 3H, (J = 6.6) Hz)</td>
</tr>
<tr>
<td>28</td>
<td>0.71 (s, 3H)</td>
</tr>
<tr>
<td>29</td>
<td>1.03 (s, 3H)</td>
</tr>
</tbody>
</table>

**\(^{13}\)C NMR**

The \(^{13}\)CNMR (CDCl\(_3\), 100Hz) spectrum of GAE\textsubscript{2} has given signal at 37.6(C-1), 32.1(C-2), 72.1(C-3), 42.4 (C-4), 141.1 (C-5), 121.3 (C-6), 31.8 (C-7,8) 50.2 (C-9), 36.6 (C-10), 21.5 (C-11), 39.9 (C-12), 42.4 (C-
121

13), 56.8 (C-14), 24.4 (C-15), 29.3 (C-16), 56.2 (C-17), 40.6(C-18),
21.7 (C-19), 138.7 (C-20), 129.6 (C-21), 46.1 (C-22), 25.4 (C-23), 12.1
(C-24), 29.6 (C-25), 20.2 (C-26) 19.8 (C-27), 18.9 (C-28), 12.2 (C-29)

GAE was found to be (13aS)-2,3,9,10-tetramethoxy-6,8,13,13a-
tetrahydro-5H-isoquinolino[2,1-b]isoquinoline i.e. Stigmasterol
(C_{29}H_{48}O)

Stigmasterol

Compound 7, GAE, a yellow crystalline solid with M.P. 276-
278°C and MW 286.23

FTIR spectra shows absorption bands at 2400 to 3400 (-OH),
1726 and 1660 cm\(^{-1}\) (C=O) and 1612, 1568 and 1506 cm\(^{-1}\) (for
aromatic C=C). The signal at S 1246 was a typical one for a C-5
hydrogen bonded hydroxyl group.

The \(^1\)H NMR spectrum of GAE showed two doublet peaks at the
region S 6.18 (\(^1\)H, H-6, J=2.3 HZ) and S 6.44 (H, H-8, J=2.4 H),
characteristics of flavonol, which contain the common 5, 7-dihydroxy
substitution pattern.

The H-6 doublet peaks occur consistently at higher field than
the signal for the H-8. In the same region of spectrum (S 6.90) another
two proton signals were also observed, which show coupling and are bonded to C-5' and C-3' atoms. These two doublet protons (3' and 5'-H) have a coupling constant of 8 HZ. A two proton doublet signal at S 8.05 (J=8 HZ) was due to H-2' and H-6'. Additionally S at 12.46, which represents four hydroxyl groups bonded with four different carbon atoms.

The above data confirmed that the \textbf{GAE}_3 may be 3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one i.e. \textbf{Kaempferol} (C_{15}H_{10}O_{6})

![Kaempferol](image)

In hot plate method, the tested extracts FCE & GAE at doses of 100,200 and 400 mg/kg exhibited 21.8% & 23.2%, 43.75% & 48.4% and 74.21% & 77.5% of inhibition respectively. In tail immersion method, withdrawal time was found to be prolonged by 90.9% & 95.3%, 122.6% & 128.1%, 127.5% & 144.5% respectively. In tail flick method, the reaction time was found to be prolonged by 48.5% & 54.5%, 69.7% & 73.3% and 75.8% & 79.4% respectively. In acetic acid induced method, the reaction time was found to be prolonged by 51.41% & 59.66%, 53.29% & 54.6% and 62.3% & 72.8% respectively.
Several acute and sub-acute tests which differ with respect to stimulus eminence, intensity and period, were employed in evaluating the analgesic effect to ascertain the analgesic properties of a substance using behavioural nociceptive tests\textsuperscript{254}. The test extracts under investigation showed a dose – dependent inhibition of pain in all the four acute pain models studied. Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure. The tested extracts showed significant effect in various acute pain models (hot plate, tail flick and tail clip tests) suggesting that the effect of these extracts on these pain models may act via centrally mediated pain control.

Anti-inflammatory activity studies were conducted by four different methods. In carrageenan induced method, the tested extracts FCE & GAE at the dose of 100, 200 and 400 mg/kg showed 31.9\% & 36.2\%, 43.8\% & 46.4\% and 50.1\% & 52.7\% respectively. In Histamine induced method, the tested extracts FCE & GAE showed 30.4\% & 35.4\%, 40.4\% & 43.2\% and 46.7\% & 46.7\% respectively. In Dextran induced method, the tested extracts FCE & GAE showed 25\% & 27.9\%, 31.3\% & 29.6\% and 35.6\% & 34.8\% respectively. In cotton pellet induced granuloma test, the tested extracts FCE & GAE showed 35.63\% & 41.22\%, 50.64\% & 51.8\% and 59.07\% & 54.27\% respectively. The abdominal writhing response involving local peritoneal receptors induced by acetic acid is a sensitive process to determine peripherally acting analgesics.
Administration of acetic acid intraperitoneally causes an increase in of PGE$_2$ and PGF$_2\alpha$ and produce algesia by inducing capillary permeability and liberating endogenous substances resembling histamine, serotonin, prostaglandins, bradykinin, and substance P that warn pain nerve endings. Acetic acid also stimulates the valinoid receptors and bradykinin B$_2$ receptors in the pathway comprising sensory afferent C-fibers. Hence, the perceived activity may be due to obstructing the synthesis or release of endogenous substances or due to desensitization of nerve fibre that carries pain sensation. The results suggest that GAE as well as FCE possess significant peripherally mediated analgesic effect. Results of Analgesic activity studies conducted by five different methods suggest that the GAE and FCE possess analgesic properties, which are mediated via peripheral and central inhibitory mechanisms. The extracts have anti-prostaglandin synthetase activity, it is conceivable that the analgesic activity of the extract may encompass, mainly, inhibition of prostaglandin amalgamation but interference of the metabolism of other algesic agents or blockade of their receptors could not be omitted. Further work is required to recognize molecular mechanism(s) underlying the analgesic activity and, too, choosiness of COX- I and II inhibitory activity of the extract.

Inhibition of carrageenan induced inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents as reported by many investigators. The sub planter injection of
carrageenan developed oedema of high intensity and persisted for 3 h after injection. The development of carrageenan induced oedema is bi-phasic where release of histamine, serotonin and kinins, are seen in first phase whereas, release of prostaglandins is observed in the second phase\textsuperscript{304, 305}. The inhibitory action of the tested extracts on carrageenan induced paw oedema in rats may be mediated through either of the mediators alone or in combination. Hence the tested extracts were further investigated against paw oedema induced by individual agents like Histamine and Dextran and showed a maximum inhibition at the dose of 400 mg/kg. The extracts at the dose of 400 mg/kg, p.o also exhibited significant anti-inflammatory effect in the cotton pellet induced granuloma test. This reflected its efficacy to a high extent to reduce an increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharide which are natural proliferative events of granulation tissue formation\textsuperscript{306, 307}. It was observed that the gain in weight of the pellets was linear with the time. This linearity was continued for eight days and then leveled off. Therefore, seven days was chosen as a convenient duration for the experiments\textsuperscript{257}. Results suggest that the GAE and FCE at doses of 100, 200 and 400 mg/kg p.o. significantly reduced the oedema produced by several inducers and are comparable with many standard drugs suggested in each model. It has been reported by many researchers that flavanoids inhibit eicosanoids synthesis by inhibiting cyclooxygenase and lipoxygenase activities\textsuperscript{258}, as well as hamper the non-enzymatic peroxidation of polyunsaturated fatty acids required
for the activation of these oxygenases. Quercetin and other flavanoids inhibit synthesis of leukotrienes and release of histamine, prostaglandins and also acts as superoxide scavengers.

Normal levels SGPT (34.22), SGOT (35.5), ALP (292.66), AST (34.21), ALT (36.75), Total Proteins (192) and bilirubin (0.29) in serum. Normal levels were found to be increased appreciably in carbon tetrachloride intoxicated control animals, and in paracetamol induced controlled animals indicating the potential inducing effect of the agents. Treatment with FCE showed decrease in serum levels by 43.1%, 55.7%, 35.3%, 67.5% respectively for SSGPT, SGOT, ALP, AST levels respectively at 200mg/kg; 76.4%, 72.8%, 66.5%, 82.3% respectively for SGPT, SGOT, ALP, AST levels respectively at 400mg/kg and treatment with GAE showed decrease in serum levels by 38.9%, 53.6%, 33.6%, 65.8% respectively for SGPT, SGOT, ALP, AST levels respectively at 200mg/kg; 70.5%, 72.0%, 62.6%, 81.4% respectively for SGPT, SGOT, ALP, AST levels respectively at 400mg/kg in CCl₄ induced model. Silimaryn, the standard drug at the dose of 50mg/kg decreased the serum levels of SGPT, SGOT, ALP, AST levels by 89.7%, 94.3%, 87.7% and 95.6% under the similar conditions. In addition appreciable increase in total protein levels were observed on administration of FCE as well as GAE in dose dependent manner.

Treatment with FCE showed decrease in serum levels by 52.9%, 40.9%, 44.9%, 63.9% respectively for SGPT, SGOT, ALP, AST levels respectively at 200mg/kg; 80.1%, 56.0%, 68.3%, 80.2% respectively for SGPT, SGOT, ALP, AST levels respectively at 400mg/kg and
treatment with GAE showed decrease in serum levels by 39.3%, 47.1%, 45.9%, 62.8% respectively for SGPT, SGOT, ALP, AST levels respectively at 200mg/kg; 83.1%, 85.2%, 78.02%, 78.3% respectively for SGPT, SGOT, ALP, AST levels respectively at 400mg/kg in CCl₄ induced model. N-acetyl-cysteine, the standard drug at the dose of 100mg/kg decreased the serum levels of SGPT, SGOT, ALP, AST levels by 93.7%, 95.7%, 85.1% and 94.2% under similar conditions. In addition appreciable increase in total protein levels were observed on administration of FCE as well as GAE in dose dependent manner.

The efficacy of any hepatoprotective drug is dependent essentially on its capability of reducing the harmful effects of hepatotoxin and in maintaining the normal hepatic physiological mechanism. Hepatoprotective activity of GAE and FCE were studied in CCl₄ and paracetamol induced liver injury in rats. Rats treated with carbon tetrachloride and paracetamol showed a significant hepatic damage as evidenced from elevated levels of hepato-specific enzymes and severe alteration in different liver parameters.

The curative efficacy of administered extracts was dose dependent as evidenced by gradual reversal of the altered values of various biochemical markers back to normal following administration. This may probably through promotional activation of antioxidative enzymes and regeneration of hepatocytes that restores the structural and functional integrity of liver. The protective effect observed on treatment with the tested extracts strongly indicates that the extract may have the ability to prevent and/or mitigate any leakages of marker enzymes keen on circulation, condition the hepatocytes to
hasten regeneration of parenchymal cells, and preserve the integrity of the plasma membranes\textsuperscript{265}.

Hepato-histological studies of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal residences and central veins. Disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty worsening were observed in paracetamol and CCl\textsubscript{4} intoxicated animals. The liver fragments of the rats treated with GAE, FCE and standard drugs followed by CCl\textsubscript{4} or paracetamol intoxication showed the absence of necrosis and vacuoles, which was a sign of protection.

The antidiabetic potential of GAE and FCE could be due to the presence of tannins, phytosterols and flavanoids which would have augmented the activity of enzymes responsible for utilization of glucose by insulin-dependent pathway. The decreased levels of lipids in serum could be due to the erratic affects of lipolytic compounds on adipose matter, majorly due to insulin. In general circumstances, insulin initiates the enzyme lipoprotein lipase, which hydrolyses triglycerides. Hyper cholesterolemia and hyper triglyceridemia caused due to inactivation of lipoprotein lipase in insulin deficiency\textsuperscript{266, 267}. The altered serum lipid profile was returned to normal after treatment with ethanol extracts. Liver and muscle glycogen contents were increased significantly over 21 days of treatment of GAE & FCE in a dose-dependent manner. From the results obtained, that both ethanol extracts viz. GAE & FCE have shown effective antidiabetic activity against streptozotocin induced diabetic wistar rats at the dose of 400
mg/kg. For comparison, FCE have exhibited a potential antidiabetic activity than GAE on long-term treatment in wistar rats. It could be due the existence of comparatively more amounts of flavonoids, tannins and phytosterols. Fever may be a result of enhanced formation of cytokines such as IL-1β, IL-6, interferons α and β and TNFα that increases the synthesis of PGE\(_2\) in hypothalamic area. Increase in cyclic AMP which in turn triggers the hypothalamus to elevate body temperature by promoting increased heat generation and decreased in heat loss. Regulation of body temperature requires a delicate balance between the production and loss of heat. Hypothalamus regulates the set point at which body temperature is maintained. GAE and FCE possess a significant antipyretic effect in the maintaining of normal body temperature and reduce yeast induced elevated rectal temperature in rats and their effect are comparable to that of standard antipyretic drug, Paracetamol. Such decreasing of rectal temperature of the tested animals may be due to the presence of a single bioactive substance or a mixture of compounds in them.

The isolation of β-sitosterol from GAE, is a plasminogen activator and promotes the formation of essential polyunsaturated fatty acids from linoleic acid, but linoleic acid is required for prostaglandin and leukotriene synthesis\(^{268}\) and thus β-sitosterol reduces prostaglandin and leukotriene synthesis. β-sitosterol possesses potent anti-inflammatory and antipyretic activity\(^{269}\) by reducing the secretion of proinflammatory cytokines and alpha-TNF\(^{270}\).