“SYNTHESIS, CHARACTERIZATION AND EVALUATION OF IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF SOME SYNTHESIZED N-[(3, 5-SUB-4, 5-DIHYDROISOXAZOL-4-YL)METHYL]ANILINE DERIVATIVES”.

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ABSTRACT
Chalcones, 1, 3-diphenylpropenones constitute one of the major classes of flavonoids. Chalcones are prepared by condensation between an Aldehyde and Ketone in the presence of sodium hydroxide was used as catalyst, the Isoxazole derivative 3,5-Substituted 4,5-dihydroisoxazole was prepared by taking 5mmol of Chalcone and 10mmol of hydroxylamine hydrochloride in presence of 12 mmol of Sodium hydroxide as catalyst. N-[(3,5-sub-4,5-dihydroisoxazol-4-y1)methyl]aniline were prepared by substituted Isoxazole derivative (0.1)mol in Methanol, Formaldehyde(0.04mol) and Aniline(0.02mol). The anti-inflammatory activity of the newly synthesized Isoxazole derivatives were carried out using Carrageenan induced rat hind paw edema method. It was observed that compounds N-[(5-(4-methoxyphenyl)-3-phenyl-4,5-dihydroisoxazol-4-y1)methyl]aniline, N-[(3-(2-fluorophenyl)-5-phenyl-4,5-dihydroisoxazol-4-y1)methyl]aniline And N-[(5-(2-fluorophenyl)-3-phenyl-4,5-dihydroisoxazol-4-y1)methyl]aniline showed significant activity 38.24, 33.8 and 39.4 % decrease in paw volume respectively at the dose of 50mg/kg body weight comparable with the standard drug Indomethacin 35.29 % decrease in paw volume at the dose 10mg/kg body weight. Other compounds showed moderate and negligible anti-inflammatory activity.
KEYWORDS: Carrageenan, Isoxazole, Chalcones, N-[(3, 5-sub-4,5-dihydroisoxazol-4-yl) methyl] aniline.

INTRODUCTION
During past decades, compounds bearing heterocyclic nuclei have received much attention due to their Chemotherapeutic value in the development of novel anti inflammatory. Anthelmintic, Anti tubercular, Anti fungal and Anti microbial activities. Chalcones, 1, 3- diphenylpropenones constitute one of the major classes of Flavonoids with widespread distribution in vegetables, fruits, tea and soy.[1,2] Prehistoric therapeutic applications of Chalcones can be associated with the thousand-year old use of plants and herbs for the treatment of different medical disorders3. Contemporary studies report a generous variation of significant pharmacological activities of Chalcones including Antiproliferative, antioxidant, anti-inflammatory and anticancer effects.[2,4, 5, 6] Chalcones are important precursors in the biosynthesis of Flavones and Flavanones and are usually synthesized from Acetophenones and Benzaldehydes via the Claisen-Schmidt condensation, using base in a polar solvent.[7- 9] In addition, more exotic synthetic protocols have been reported, such as the palladium-mediated Suzuki coupling between Cinnamoyl chloride and Phenylboronic acids or the Carbonylative heck coupling with aryl halides and Styrenes in the presence of Carbon monoxide.[10, 11] The main objective of this research work was to synthesize, purify and evaluate Anti-inflammatory activity using Carrageenan induced rat hind paw edema method of the newly synthesized Isoxazoline derivatives.

MATERIALS AND METHODS
1. Chemicals used: focus
All the chemicals used in the present project work were of AR grade and LR grade, purchased from S-D Fine Loba Chemie, Qualigens, and Merck.

2. Synthesis of Chalcones[12-14]
Chalcones can be prepared by condensation between an Aldehyde and an Ketone in the presence of sodium hydroxide as a catalyst. (0.55)mol of Sodium hydroxide was dissolved in 160ml of water and 120ml of absolute alcohol in a beaker kept in ice bath. (0.43)mmol of aldehyde and (0.43)mmol of Ketone were added drop wise with stirring. The stirring is continued till the formation of precipitate, then filtered off to get Chalcone. The obtained product is recrystallised from alcohol.
IR [KBr] cm\(^{-1}\): 1658 (C=O), 1589, (C=C), 678 (C–Cl).
\(^1\)H NMR (CDCl\(_3\)): □ 7.93-8.2(m, Ar-H), 7.64(d, COCH=CH), 7.5(d, COCH=CH).

3. Preparation of 3,5-Substituted 4,5-dihydroisoxazole.\(^{[15,16]}\)
3,5-Substituted 4,5-dihydroisoxazole was prepared by taking 5mmol of Chacone obtained from the previous step and 10mmol of hydroxylamine hydrochloride in presence of 12mmol of sodium hydroxide as catalyst. The mixture was refluxed for 10 to 12 hrs on a heating mantle by using alcohol as solvent. After refluxing the mixture is poured onto ice cold water to get precipitate. Then it is filtered off, washed with water and recrystallised from alcohol. IR [KBr] cm\(^{-1}\): 1595 (C=N), 1348(C–O–N), 692(C–Cl).
\(^1\)H NMR (CDCl\(_3\)): δ 7.3–7.46(m, Ar-H), 4.28 (dd, 1H isoxazoline), 3.5 (dd, 1H isoxazoline).

4. Preparation of \(N\)-[(3,5-sub-4,5-dihydroisoxazol-4-yl)methyl]aniline.\(^{[15,16]}\)
To the prepared substituted isoxazole derivative (0.1)mol in methanol, Formaldehyde(0.04mol) and Aniline(0.02mol) were added dropwise. The reaction mixture was refluxed for 2hr, then the excess solvent is distilled off and poured into ice water, Solid obtained is filtered, washed with water and recrystallised from chloroform. IR [KBr] cm\(^{-1}\): 3215 (NH stretch), 1593(C=N), 1336(C-O-N), 763(C-Cl). \(^1\)H NMR (CDCl\(_3\)): δ 7.7(t, 2H CHCH\(_2\)), 7.1-7.4 (m, Ar, H), 4.0-5.0(m, NH).
5. Anti Inflammatory Studies.\cite{17,18}

The anti-inflammatory activity of the newly synthesized Isoxazole derivatives were carried out using Carrageenan induced rat hind paw edema method. The adult Wistar albino rats of either sex weighing 125-150 g were selected and were assigned into ten groups of 6 animals each. They were marked with picric acid solution for individual animal identification. The animals were deprived of food overnight. (Water ad libitum) and the synthetic compounds were administered once before the injection of Carrageenan. The first group served as solvent control received normal saline, Second group served as positive control, received Indomethacin (10mg/kg), suspended in 0.5% w/v Carboxy methyl cellulose sodium salt, orally. The remaining eight groups (3\textsuperscript{rd} -10) are administered with synthesized (N-[(3,5-sub-4,5-dihydroisoxazol-4-yl)methyl]aniline) derivatives at dose level of 50mg/kg suspended in 0.5% w/v carboxy methyl cellulose sodium salt, in a volume not exceeding 0.1ml/100gms orally. After 30 min of test compound administration 0.1ml of 1% w/v of carrageenan in normal saline was injected into the subplantar region of the left hind paw of the rats of all groups. Immediately after the carrageenan injection, the volume of its displacement was measured using plethesmometer.

The reading was recorded at the end of 180 min. The % inhibition of edema was calculated by using the formula.

\[
\% \text{ Inhibition} = 100 \left(1 - \frac{V_t}{V_l}\right).
\]

\(V_t/VI\) = Edema volume in the rat treated with test drugs and control respectively.

RESULTS

1. 3,5-Substituted 4,5-dihydroisoxazole.

3,5-Substituted 4,5-dihydroisoxazole was prepared by taking 5mmol of chalcone and 10mmol of hydroxylamine hydrochloride in presence of 12 mmol of sodium hydroxide as catalyst. 3,5-Substituted4,5-dihydroisoxazole list of compounds were given in table no.1.
2. \textit{N-[(3,5-sub-4,5-dihydroisoxazol-4-yl)methyl]aniline.}

\textit{N-[(3,5-sub-4,5-dihydroisoxazol-4-yl)methyl]aniline} were prepared by substituted isoxazole derivative (0.1)mol in methanol, Formaldehyde(0.04mol) and Aniline(0.02mol), list of compounds were given in table no.2.

3. \textbf{Anti Inflammatory Studies.}

The anti-inflammatory activity of the newly synthesized Isoxazole derivatives were carried out using carrageenan induced rat hind paw edema method. The list of compounds and their anti-inflammatory activity were given in table no.3.

\textbf{Table no. 1. List of synthesized 3,5 Substituted4,5-dihydroisoxazole derivatives}

<table>
<thead>
<tr>
<th>Isoxazole</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>IUPAC name</th>
<th>% yield</th>
<th>Mobile phase</th>
<th>Melting Point in °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C_{15}H_{13}NO</td>
<td>222.2</td>
<td>3,5-diphenyl-4,5-dihydroisoxazole.</td>
<td>59</td>
<td>Hexane:EA 3:2 +1drop CH$_3$COOH</td>
<td>118-120</td>
</tr>
<tr>
<td>2</td>
<td>C_{15}H_{12}ClNO</td>
<td>257.7</td>
<td>5-(3-chlorophenyl)-3-phenyl-4,5-dihydroisoxazole.</td>
<td>46</td>
<td>„</td>
<td>196-198</td>
</tr>
<tr>
<td>3</td>
<td>C_{16}H_{14}ClNO$_2$</td>
<td>287.7</td>
<td>5-(3-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazole.</td>
<td>48</td>
<td>„</td>
<td>140-142</td>
</tr>
<tr>
<td>4</td>
<td>C_{16}H_{15}NO$_2$</td>
<td>253.2</td>
<td>3-(4-methoxyphenyl)-5-phenyl-4,5-dihydroisoxazole.</td>
<td>54</td>
<td>„</td>
<td>158-160</td>
</tr>
<tr>
<td>5</td>
<td>C_{15}H_{11}Cl$_2$NO</td>
<td>292.1</td>
<td>3-(2,4-dichlorophenyl)-5-phenyl-4,5-dihydroisoxazole.</td>
<td>62</td>
<td>„</td>
<td>114-116</td>
</tr>
<tr>
<td>6</td>
<td>C_{15}H_{12}FNO</td>
<td>241.2</td>
<td>3-(2-fluorophenyl)-5-phenyl-4,5-dihydroisoxazole.</td>
<td>50</td>
<td>„</td>
<td>182-184</td>
</tr>
<tr>
<td>7</td>
<td>C_{15}H_{10}Cl$_3$NO</td>
<td>326.60</td>
<td>5-(3-chlorophenyl)-3-(2,4-dichlorophenyl)-4,5-dihydroisoxazole.</td>
<td>51</td>
<td>„</td>
<td>196-198</td>
</tr>
<tr>
<td>8</td>
<td>C_{15}H_{11}CIFNO</td>
<td>275.7</td>
<td>5-(3-chlorophenyl)-3-(2-fluorophenyl)-4,5-dihydroisoxazole.</td>
<td>45</td>
<td>„</td>
<td>242-244</td>
</tr>
</tbody>
</table>
Table no. 2. List of synthesized \(N-[(3,5\text{-sub-4,5-dihydroisoxazol-4-yl})\text{methyl}]\)aniline derivatives.

<table>
<thead>
<tr>
<th>Mannich product</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>IUPAC name</th>
<th>% yield</th>
<th>Mobile phase</th>
<th>Melting Point in °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(\text{C}<em>{22}\text{H}</em>{20}\text{N}_{2}\text{O})</td>
<td>328.4</td>
<td>(N-[(3,5\text{-diphenyl-4,5-dihydroisoxazol-4-yl})\text{methyl}])aniline.</td>
<td>44</td>
<td>Petether :DCM 2:2+1drop CH(_3)COOH</td>
<td>138-140</td>
</tr>
<tr>
<td>2</td>
<td>(\text{C}<em>{22}\text{H}</em>{19}\text{ClN}_{2}\text{O})</td>
<td>362.8</td>
<td>(N-[(5-(3\text{-chlorophenyl})-3\text{-phenyl-4,5-dihydroisoxazol-4-yl})\text{methyl}])aniline.</td>
<td>39</td>
<td>&quot;</td>
<td>158-160</td>
</tr>
<tr>
<td>3</td>
<td>(\text{C}<em>{23}\text{H}</em>{21}\text{ClN}<em>{2}\text{O}</em>{2})</td>
<td>392.8</td>
<td>(N-[(5-(3\text{-chlorophenyl})-3-(4\text{-methoxyphenyl})-4,5\text{-dihydroisoxazol-4-yl})\text{methyl}])aniline.</td>
<td>52</td>
<td>&quot;</td>
<td>214-216</td>
</tr>
<tr>
<td>4</td>
<td>(\text{C}<em>{23}\text{H}</em>{22}\text{N}<em>{2}\text{O}</em>{2})</td>
<td>358.4</td>
<td>(N-[(5-(4\text{-methoxyphenyl})-3\text{-phenyl-4,5-dihydroisoxazol-4-yl})\text{methyl}])aniline.</td>
<td>40</td>
<td>&quot;</td>
<td>96-98</td>
</tr>
<tr>
<td>5</td>
<td>(\text{C}<em>{22}\text{H}</em>{18}\text{Cl}<em>{2}\text{N}</em>{2}\text{O})</td>
<td>397.2</td>
<td>(N-[(3-(2,4\text{-dichlorophenyl})-5\text{-phenyl-4,5-dihydroisoxazol-4-yl})\text{methyl}])aniline.</td>
<td>38</td>
<td>&quot;</td>
<td>144-146</td>
</tr>
<tr>
<td>6</td>
<td>(\text{C}<em>{22}\text{H}</em>{19}\text{FN}_{2}\text{O})</td>
<td>346.3</td>
<td>(N-[(5-(2\text{-fluorophenyl})-3\text{-phenyl-4,5-dihydroisoxazol-4-yl})\text{methyl}])aniline</td>
<td>44</td>
<td>&quot;</td>
<td>160-162</td>
</tr>
<tr>
<td>7</td>
<td>(\text{C}<em>{22}\text{H}</em>{17}\text{ClN}_{2}\text{O})</td>
<td>431.7</td>
<td>(N-[(5-(3\text{-chlorophenyl})-3\text{-(2,4-dichlorophenyl)-4,5-dihydroisoxazol-4-yl})\text{methyl}])aniline.</td>
<td>32</td>
<td>&quot;</td>
<td>98-100</td>
</tr>
<tr>
<td>8</td>
<td>(\text{C}<em>{22}\text{H}</em>{18}\text{ClF}<em>{2}\text{N}</em>{2}\text{O})</td>
<td>380.8</td>
<td>(N-[(3-(4\text{-chlorophenyl})-5\text{-fluorophenyl})-4,5\text{-dihydroisoxazol-4-yl})\text{methyl}])aniline</td>
<td>49</td>
<td>&quot;</td>
<td>253-255</td>
</tr>
</tbody>
</table>
The following $N$-[(3,5-sub-4,5-dihydroisoxazol-4-y)-methyl]aniline derivatives were prepared:

1. $N$-[(3,5-Diphenyl-4,5-dihydroisoxazol-4-yl)methyl]aniline

2. $N$-[(3-(4-Chlorophenyl)-5-phenyl-4,5-dihydroisoxazol-4-yl)methyl]aniline

3. $N$-[(3-(4-Chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydroisoxazol-4-yl)methyl]aniline

4. $N$-[(5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydroisoxazol-4-yl)methyl]aniline
(5) \(N\)-\{5-(2,4-Dichlorophenyl)-3-phenyl-4,5-dihydroisoxazol-4-yl\}methyl\:aniline

(6) \(N\)-\{5-(2-Fluorophenyl)-3-phenyl-4,5-dihydroisoxazol-4-yl\}methyl\:aniline

(7) \(N\)-\{3-(4-Chlorophenyl)-5-(2,4-dichlorophenyl)-4,5-dihydroisoxazol-4-yl\}methyl\:aniline

(8) \(N\)-\{3-(4-Chlorophenyl)-5-(2-fluorophenyl)-4,5-dihydroisoxazol-4-yl\}methyl\:aniline
Table no. 3. Effect of Isoxazole Derivatives on Carrageenan Induced Paw Edema in Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Increased paw volume after 30 min</th>
<th>% decrease in paw volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>2ml/kg</td>
<td>0.64±0.04</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>Indomethacin</td>
<td>10</td>
<td>0.44±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.29</td>
</tr>
<tr>
<td>3</td>
<td>N-[(3,5-diphenyl-4,5-dihydroisoxazol-4-yl)methyl]aniline</td>
<td>50</td>
<td>0.59±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.24</td>
</tr>
<tr>
<td>4</td>
<td>N-[[5-(3-chlorophenyl)-3-phenyl-4,5-dihydroisoxazol-4-yl]methyl]aniline</td>
<td>50</td>
<td>0.53±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.06</td>
</tr>
<tr>
<td>5</td>
<td>N-benzyl-3-(4-methoxyphenyl)-5-phenyl-4,5-dihydroisoxazol-4-yl]methyl]aniline</td>
<td>50</td>
<td>0.54±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.59</td>
</tr>
<tr>
<td>6</td>
<td>N-[[5-(4-methoxyphenyl)-3-phenyl-4,5-dihydroisoxazol-4-yl]methyl]aniline</td>
<td>50</td>
<td>0.42±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.24</td>
</tr>
<tr>
<td>7</td>
<td>N-[[3-(2-fluorophenyl)-5-phenyl-4,5-dihydroisoxazol-4-yl]methyl]aniline</td>
<td>50</td>
<td>0.45±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.8</td>
</tr>
<tr>
<td>8</td>
<td>N-[[5-(2-fluorophenyl)-3-phenyl-4,5-dihydroisoxazol-4-yl]methyl]aniline</td>
<td>50</td>
<td>0.48±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.4</td>
</tr>
<tr>
<td>9</td>
<td>N-[[5-(3-chlorophenyl)-3-(2,4-dichlorophenyl)-4,5-dihydroisoxazol-4-yl]methyl]aniline</td>
<td>50</td>
<td>0.52±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.53</td>
</tr>
<tr>
<td>10</td>
<td>N-[[3-(4-chlorophenyl)-5-(2-fluorophenyl)-4,5-dihydroisoxazol-4-yl]methyl]aniline</td>
<td>50</td>
<td>0.54±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.59</td>
</tr>
</tbody>
</table>

(number of animals)=6, values were expressed as mean ±SEM.

<sup>a</sup>p<0.001;  <sup>b</sup>p<0.01;  <sup>c</sup>p <0.01 vs control.  <sup>d</sup>p<0.05 vs Indomethacin.

Values were analysed one way Anova followed by Tukey multiple comparision test.

DISCUSSION

Isoxazole differs from pyridine in undergoing more readily Electrophilic substitution reactions and possessing a more liable ring; this relationship thus resembles that between furan and benzene.<sup>19</sup> Isoxazole derivatives are a class of heterocyclic compounds, are reported to show potent anti-tuberculosis<sup>20</sup>, anti-microbial<sup>21</sup> and anti-inflammatory<sup>22</sup> activities. These interesting pharmacological properties exhibited by Isoxazolines have prompted us to synthesize some novel Isoxazoline derivatives and the so synthesized compounds were further screened for their anti-inflammatory activity. The titled compounds were synthesized according to the procedures as given in the methodology. The reactions were monitored by TLC. The physical constants like melting point and solubility were determined for all the intermediate and final products. All the titled compounds were evaluated for their anti-inflammatory activity. Edema which is developed after carragenin injection is a biphasic event. The initial phase is attributed to the release of histamine and...
serotonin. The edema maintained between the 1\textsuperscript{st} and 2\textsuperscript{nd} phase is due to kinin like substances. The second phase is said to be promoted by prostaglandin. It is reported that the second phase of edema is sensitive to drugs like hydrocortisone, Phenylbutazone and Indomethacin.

It was observed that compounds $N$-\{\[5-(4-methoxyphenyl)-3-phenyl-4,5-dihydroisoxazol-4-yl\]methyl\}aniline, $N$-\{\[3-(2-fluorophenyl)-5-phenyl-4,5-dihydroisoxazol-4-yl\]methyl\}aniline and $N$-\{\[5-(2-fluorophenyl)-3-phenyl-4,5-dihydroisoxazol-4-yl\]methyl\}aniline showed significant activity 38.24, 33.8 and 39.4 % decrease in paw volume respectively at the dose of 50mg/kg body weight comparable with the standard drug Indomethacin 35.29 % decrease in paw volume at the dose 10mg/kg body weight. Other compounds showed moderate and negligible anti-inflammatory activity.

**CONCLUSION**

The yield of the synthesized compounds was found to be in range from 32%- 52%. A single method used to perform the anti-inflammatory activity using carrageenan induced paw edema in rats. Most of the compounds tested showed good anti-inflammatory activity at the concentration of 50 mg/kg using Indomethacin as standard. Among the synthesized compounds, $N$-\{\[5-(4-methoxyphenyl)-3-phenyl-4,5-dihydroisoxazol-4-yl\]methyl\}aniline,$N$-\{\[3-(2-fluorophenyl)-5-phenyl-4,5-dihydroisoxazol-4-yl\]methyl\}aniline and $N$-\{\[5-(2-fluorophenyl)-3-phenyl-4,5-dihydroisoxazol-4-yl\]methyl\}aniline showed strong anti-inflammatory activity the remaining compounds exhibited mild to moderate activity. Hence, newly synthesized isoxazole derivatives do possess considerable anti-inflammatory activity and further lead optimization should be carried out for the better-expected anti-inflammatory activity.

**REFERENCE**

