Modulation by insulin rather than blood glucose of the pain threshold in acute physiological and flavone induced antinociception in mice

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The present study investigated the cause effect relationship between glycemic and algesic states. The hypo- and hyperglycemic conditions were induced physiologically through exercise (3 min swim at room temperature 28°-30°C) and external dextrose (2 g/kg, ip) administration respectively in mice. Besides, flavone (50 mg/kg, sc) a known antinociceptive drug was chosen to study such a cause effect relationship. The anti-nociception was assessed by acetic acid assay, blood glucose measured using glucometer (Ames) and serum insulin by radioimmunoassay. The findings revealed that irrespective of the glycemic state whether hypo-, hyper-, or euglycemic induced by swim stress, dextrose or flavone per se respectively, significant antinociceptive response was recorded. Pretreatment with flavone (50 mg/kg, sc) always exhibited a tendency to reverse the hyperglycemia, if any, but enhanced the antinociceptive response either after swim stress or after dextrose. These data support the contention that changes in the glycemic state in acute condition is not responsible for antinociceptive response and thereby suggesting dissociation between these two parameters. Extended studies estimating serum insulin level after the above mentioned maneuvers showed a significant rise whenever antinociceptive response was recorded irrespective of the glycemic state. It is suggested that serum insulin level, a hormonal parameter rather than the blood glucose level, which is a metabolic parameter, appears more reliable. It appears that the changes in serum insulin level produced by various treatments may have a relationship with the antinociceptive response. However, this study has the limitation that the results can apply only for acute conditions and extrapolation to clinical conditions is debatable.

The management of diabetic neuropathy, a major problem, has stimulated the researchers to identify effective analgesics for use in this situation. Many scientists have attempted to correlate or associate the changes in blood glucose level and pain threshold. Davis et al.1 were the first to communicate a relationship between insulin and antinociceptive action and that insulin induced hypoglycemia enhanced the morphine induced antinociception in rats. Whereas the exogenous administration of glucose potentiated morphine antinociception2 as reduced sensitivity to morphine in streptozotocin as well as genetically induced diabetic animals has also been reported3. Clinical studies reporting hyperalgesic state in either manipulated glycemic state in healthy volunteers or in diabetic patients lead to the notion that a correlation between algesic and glycemic states existed4-5. However, these findings were not reproducible by another group of workers6-7. A recent report8 showing an inherent antinociceptive response for insulin without any hypoglycemic effect when administered intracerebroventricularly (icv) suggested an extra metabolic antinociception function for insulin which might play a key role wherever algesic and glycemic changes are anticipated.

While reviewing the extensive literature available on these lines it was found that most of the available information is obtained from experimental diabetic animal models or from diabetic patients.

Diabetes mellitus (DM) either induced chemically in animals or considered as a disease in humans has been shown to induce structural as well as functional changes in the neuronal system. Histopathological studies have demonstrated a neuronal degeneration in streptozotocin or alloxan induced animal models possibly by altering the neurotransmitters’ function9, vasoactive intestinal polypeptides10, modification of the metabolic profile of arachidonic acid11 or by increasing the activity of calcium channels12. Circulating endogenous opioid peptide levels were found to be significantly reduced in these diabetic animals13.
Based on these reports it is possible that algesic state either through opioidergic system or through arachidonic pathway is likely to be modified by streptozotocin or alloxan. Therefore, the measurement of pain threshold employing any experimental diabetic model in these animals might not have reflected the true pain threshold. This must be the reason for the varied results observed by different workers in these lines.

Considering these aspects we proposed to approach this problem using physiological models where the neuronal structure and functions are expected to be normal and are protected. As a first step we investigated experimentally the possible association between acute changes in glycemic state achieved through physiological means and pain threshold level in healthy animals. Flavonoid group of substances has been shown to elicit opioid mediated antinociceptive response in experimental models. Therefore, the basic nucleus of this group, flavone was examined for the said investigation since minimal untoward responses are expected with this compound. Additionally, the possible mechanism operating in such change was also investigated.

Materials and Methods

Animals—Adult male Swiss albino mice (King Institute, Madras, India) weighing 20-25 g were used in this study. They were housed in polypropylene cages in group of six and had free access to food (pellets obtained from Gold Mohar Ltd., Bangalore, India) and water until the initiation of the experimental procedure. They were maintained under normal room temperature (28°-30°C) with 12:12 hr light: dark cycle. The experiment was conducted during the light period.

Drugs and Chemicals—Flavone (Herborgonics, Madras, India), glacial acetic acid A.R. (Sarabhai, Vadodhora, India), naloxone hydrochloride (Endo Laboratories, NY, USA), dextrose I.P. (Indian Drugs and Pharmaceuticals Ltd., Hyderabad, India), insulin radio-immunoassay kit (Diagnostic Products Corporation, Los Angeles, USA), and carboxy methyl cellulose sodium salt (Glaxo Laboratories, Bombay, India) were used.

Flavone was used as a suspension in saline using 1% carboxy methylcellulose. Naloxone hydrochloride and dextrose were dissolved in saline. Appropriate vehicle treated animal group was included to obtain control data.

Induction of swim stress antinociception—intraperitoneal administration of 2 g/kg of dextrose (25% solution) produced significant hyperglycemia, which can be quantified in the adopted procedure. Therefore, this concentration was used for further studies. Antinociceptive response was assessed in these animals 15 min after dextrose injection. The presence of hyperglycemia was confirmed by meas-

Assessment of antinociception—The antinociceptive response was evaluated by the acetic acid induced abdominal constriction test. Mice were injected 10 ml/kg of 0.6% freshly prepared acetic acid intraperitoneally. The number of abdominal constrictions following this injection for a period of 30 min was recorded. A significant reduction (P < 0.05) in the number of abdominal constrictions as compared to vehicle treated mice was considered as antinociceptive response. The assessment of antinociception was blinded to avoid the bias.

Estimation of blood glucose—The blood glucose was measured using Ames glucometer and glucostix (Bayers Diagnostics, Mumbai, India). A drop of blood from the animal was collected by cutting the tip of its tail, allowing it to react with the appropriate glucostix and the glucose levels measured. The values were compared by auto-analyzer method by collecting another sample from the same animal. The measurement of blood glucose in the experimental animal was made prior to the exposure of any drug/stress and then just before the measurement of antinociception. The results were expressed as percentage change in the blood glucose considering the initial blood glucose value of that animal as 100%.

Physiological manoeuvres / Drug treatment

Induction of hypoglycemia—The swim stress employed to induce antinociception was also found to elicit hypoglycemia in our pilot study. Therefore, another method was not required besides this physiological stress to induce hypoglycemia.

Induction of hyperglycemia—Exogenous administration of dextrose was employed for this purpose. Pilot studies conducted in our laboratory revealed that intraperitoneal administration of 2 g/kg of dextrose (25% solution) produced significant hyperglycemia, which can be quantified in the adopted procedure. Therefore, this concentration was used for further studies. Antinociceptive response was assessed in these animals 15 min after dextrose injection. The presence of hyperglycemia was confirmed by meas-
uring blood glucose level in these animals just before acet ic acid challenge.

**Drug treatment**

*Flavone treatment*—Mice received different doses of flavone (6.25, 12.5, 25, 50 and 100 mg/kg; sc) 60 min prior to acet ic acid challenge and the antinociception was assessed. From the results, the effective dose of flavone 50 mg/kg was selected for further studies. The blood glucose level was measured prior to and 60 min after flavone (50 mg/kg; sc) administration.

*Flavone-swim stress treatment*—The influence of flavone on swim stress induced changes in blood glucose and antinociception was measured by exposing the animals to flavone (50 mg/kg; sc) 60 min prior to swim stress. The parameters were assessed as described above.

*Flavone-dextrose treatment*—A group of animals, which received flavone (50 mg/kg; sc), were also treated with dextrose (2 g/kg; ip) 45 min after flavone and then subjected to antinociceptive assay 15 min later. Blood glucose levels in these animals were also measured just before flavone administration and prior to acet ic acid challenge.

*Flavone-dextrose-swim stress treatment*—A separate group of animals were exposed to flavone (50 mg/kg; sc) and also administered dextrose (2 g/kg; ip) 45 min later and then subjected to swim stress 15 min after dextrose. The antinociceptive assay was assessed immediately after swim stress. The blood glucose levels were measured as above.

**Role of opioid system**—The role of opioid system in the possible changes induced by flavone, swim stress, flavone-swim stress, flavone-dextrose, or flavone-dextrose-swim stress treatment on the blood glucose and antinociception was investigated by treating the animals with naloxone. Animals receiving various treatments described above were injected with naloxone (5 mg/kg; ip) 10 min prior to swim stress or acet ic acid challenge and the antinociceptive response recorded. In these animals, blood glucose was recorded before the beginning of the experiment and just prior to acet ic acid challenge.

**Estimation of insulin**—The possible role of insulin which controls the blood glucose levels, in the antinociceptive response and in the association if any, between blood glucose changes and antinociception sought after, was investigated by measuring the serum insulin level using a insulin radio-immunoassay kit (Diagnostic Products Corporation, Log Angeles, USA) and a gamma counter (RIASTAR, 5405, PACKARD, USA).

Serum sample (200 μL) was stored in a refrigerator at 4°C until initiation of radioimmunoassay on the subsequent day. Before assay, the samples were allowed to return to room temperature (28°C) and mixed by gentle inversion.

**Statistical analysis**—The data were subjected to ANOVA followed by Dunnett’s ‘t’ test. P < 0.05 was considered as statistically significant response (F = 278; df = 40). Wherever pertinent, correlation coefficient was calculated especially between blood glucose, insulin level and anti-nociception.

**Results**

**Effect of flavone on antinociception in mice**—Flavone in varying doses, elicited a significant dose-dependent antinociception as evidenced by an increase in the percent inhibition of acet ic acid induced abdominal constrictions as compared to vehicle tested mice. An effective and marked inhibition was observed with 50 mg/kg of flavone beyond which there was no further significant increase in inhibition of abdominal constrictions (Fig.1).

**Effect of flavone on blood glucose, serum insulin levels and antinociception in mice**—Flavone (50 mg/kg, sc) elicited significant antinociceptive response with mild but insignificant reduction in the blood glucose level as compared with vehicle treated mice (Table 1). Conversely, there was an increase in serum insulin level after flavone administration which was statistically significant (P < 0.02) as compared to vehicle treated mice (Table 1).

**Effect of swim stress on blood glucose, serum insulin levels and antinociception in mice**—Swim stress produced a marked inhibition of abdominal constrictions associated with a reduction in blood glucose level and an elevation in serum insulin level (Table 1). These changes (vehicle vs swim stress) were statistically significant (P < 0.001). When compared with flavone treatment, the changes induced by swim stress in blood glucose and serum insulin level and inhibition of abdominal constrictions were significantly higher (P < 0.001; Table 1).

**Effect of flavone treatment on swim stress induced changes in blood glucose, serum insulin levels and antinociception**—Flavone pretreatment did not modify either the significant hypoglycemia or the elevated serum insulin level elicited by swim stress. The changes in blood glucose and serum insulin levels (swim stress vs flavone-swim stress) were not statisti-
Effect of dextrose on blood glucose, serum insulin levels and antinociception in mice—Administration of dextrose (2 g/kg; ip.) elevated the blood glucose as well as serum insulin levels associated with marked inhibition of abdominal constrictions when compared with vehicle treated mice. This increase in all the three responses (vehicle vs. dextrose) was statistically significant ($P < 0.001$). The blood glucose and serum insulin levels as well as the inhibition of abdominal constrictions produced by dextrose were much higher than those produced by either flavone or swim stress alone. These changes (dextrose vs flavone or swim stress) were statistically significant ($P < 0.001$; Table 1).

Effect of flavone on dextrose induced changes in blood glucose, serum insulin levels and antinociception—Flavone administration attenuated dextrose induced hyperglycemia which was otherwise marked in animals, which did not receive flavone. This attenuation was statistically significant ($P < 0.01$). Though dextrose produced a mild increase in serum insulin level in flavone treated animals it was not statistically significant (compare dextrose vs flavone-dextrose). However, there was no statistically significant difference in the antinociceptive response induced by either dextrose or flavone-dextrose treatment. Comparing flavone vs flavone-dextrose treatment, dextrose administration resulted in hyperglycemia as well as hyperinsulinemia associated with potentiated antinociceptive response. All these findings were statistically significant ($P < 0.001$; Table 1).

Effect of dextrose on swim stress induced changes in blood glucose, serum insulin levels and antinociception—Dextrose treatment reversed the hypoglycemia induced by swim stress and elicited hyperglycemia. A maximum increase in serum insulin level was noted in animals that were subjected to swim stress after dextrose treatment. These effects were statistically significant ($P < 0.001$). The swim stress induced antinociceptive response was further potentiated almost near to 100% inhibition in dextrose treated animals. This potentiated antinociception (swim stress vs dextrose-swim stress) was statistically significant ($P < 0.001$). Comparison of the effects produced by swim stress, dextrose and dextrose-swim stress treatments shows that there is a graded increase in the antinociceptive response as the serum insulin levels and antinociception induced by flavone, swim stress, dextrose or naloxone alone or in combination in mice

Table 1 — Changes in blood glucose, serum insulin levels and antinociception induced by flavone, swim stress, dextrose or naloxone alone or in combination in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood glucose μM</th>
<th>Serum insulin μU/mL</th>
<th>Percent inhibition of abdominal constriction after 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>100.4 ± 5.6</td>
<td>21.5 ± 0.7</td>
<td>—</td>
</tr>
<tr>
<td>2 Flavone (Fla)</td>
<td>92.4 ± 10.3</td>
<td>24.1 ± 0.5</td>
<td>—</td>
</tr>
<tr>
<td>Swim stress (SS)</td>
<td>65.3 ± 7.9h</td>
<td>32.8 ± 1.6</td>
<td>67.7 ± 0.9</td>
</tr>
<tr>
<td>Flavone + SS</td>
<td>64.9 ± 6.7h</td>
<td>33.8 ± 0.9</td>
<td>80.7 ± 1.0</td>
</tr>
<tr>
<td>3 Dextrose (Dex)</td>
<td>250.5 ± 16.5g</td>
<td>43.2 ± 2.4</td>
<td>77.3 ± 0.4</td>
</tr>
<tr>
<td>Flavone + Dextrose</td>
<td>154.6 ± 15.8g</td>
<td>44.6 ± 1.2</td>
<td>78.6 ± 1.0</td>
</tr>
<tr>
<td>Dextrose + SS</td>
<td>148.4 ± 9.9g</td>
<td>105.8 ± 4.4</td>
<td>97.2 ± 0.5</td>
</tr>
<tr>
<td>Flavone + Dextrose + SS</td>
<td>84.4 ± 2.6g</td>
<td>38.5 ± 1.9</td>
<td>97.9 ± 0.5</td>
</tr>
<tr>
<td>4 Naloxone (Nal)</td>
<td>1053.5 ± 4.2</td>
<td>21.5 ± 1.3</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>Flavone + Dex + Nal + SS</td>
<td>142.4 ± 11.2b</td>
<td>38.8 ± 1.3</td>
<td>97.9 ± 0.5</td>
</tr>
</tbody>
</table>

1 Blood glucose was expressed as percentage considering the initial value as 100%
2 Flavone 50 mg/kg was administered sc 60 min prior to swim stress or aceitic acid challenge
3 Dextrose 2 g/kg was administered ip 15 min prior to swim stress or aceitic acid challenge
4 Naloxone 5 mg/kg was administered ip 10 min prior to swim stress or aceitic acid challenge
5 $P < 0.001$, $P < 0.01$, $P < 0.02$, $P < 0.05$ compared to vehicle treatment
6 $P < 0.001$ compared to flavone treatment
7 $P < 0.001$ compared to swim stress treatment
8 $P < 0.001$ compared to flavone or swim stress treatment
9 $P < 0.01$ compared to dextrose treatment
10 $P < 0.001$ compared to flavone treatment
11 $P < 0.001$ compared to dextrose treatment
12 $P < 0.001$ compared to flavone treatment
13 $P < 0.001$ compared to dextrose treatment
14 $P < 0.001$ compared to Flavone + SS treatment
15 $P < 0.001$ compared to Dextrose + SS treatment
16 $P < 0.001$ compared to Flavone + Dextrose + SS treatment
17 $P < 0.001$ compared to Flavone + Dextrose + SS treatment
level increases and this effect is independent of the glycermic state, either hypo or hyperglycemia (Table 1).

Effect of flavone on dextrose induced changes in blood glucose, serum insulin levels and antinociception in mice — Dextrose administration attenuated the hypoglycemia induced by flavone-swim stress treatment and elevated the blood glucose level but yet maintained the hypoglycemic state as compared to vehicle treated mice. This change (flavone-swim stress vs flavone-dextrose-swim stress) was statistically significant (\( P < 0.05 \)). The serum insulin level was increased by dextrose in this group of animals and this effect was statistically significant (\( P < 0.05 \)). In these animals, the antinociceptive response also was near to 100%. This potentiation was statistically significant (\( P < 0.001 \)). Comparing dextrose-swim stress vs flavone-dextrose-swim stress treatments, it is interesting to note that flavone administration reversed the hyperglycemia as well as hyperinsulinemia and these reversals were statistically significant (\( P < 0.001 \)). However, there was no statistically significant difference in the potentiated antinociceptive response between these two treatment groups of animals. The influence of dextrose treatment on changes in blood glucose, serum insulin levels and antinociceptive response appear to be more predominant than that produced by flavone in the stressed mice (Tables 1).

Role of opioid system

Effect of naloxone on blood glucose, serum insulin levels and antinociception in mice — Naloxone (5 mg/kg, ip) did not produce any statistically significant

Table 2 — Effect of naloxone on changes in blood glucose level and antinociception induced by flavone, swim stress or dextrose alone or in combination in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood glucose</th>
<th>Percent inhibition of abdominal constrictions after 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>100.4 ± 5.6</td>
<td>—</td>
</tr>
<tr>
<td>^2Naloxone (Nal)</td>
<td>105.3 ± 4.2</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>^3Flavone (Fla)</td>
<td>92.4 ± 10.3</td>
<td>55.0 ± 2.0^a</td>
</tr>
<tr>
<td>Fla + Nal</td>
<td>94.4 ± 4.3</td>
<td>1.2 ± 0.9^b</td>
</tr>
<tr>
<td>Swim stress (SS)</td>
<td>65.3 ± 7.9^a</td>
<td>67.7 ± 0.9^b</td>
</tr>
<tr>
<td>^4Dextrose</td>
<td>250.4 ± 14.2^a</td>
<td>77.3 ± 1.2^a</td>
</tr>
<tr>
<td>^4Dextrose + swim Stress</td>
<td>148.4 ± 9.8^a</td>
<td>97.2 ± 0.9^b</td>
</tr>
<tr>
<td>Nal + SS</td>
<td>80.7 ± 8.1</td>
<td>51.1 ± 2.1^ad</td>
</tr>
<tr>
<td>Fla + SS</td>
<td>64.9 ± 6.7^a</td>
<td>80.7 ± 1.0^b</td>
</tr>
<tr>
<td>Fla + Nal + SS</td>
<td>58.5 ± 2.8^a</td>
<td>67.5 ± 2.3^a</td>
</tr>
<tr>
<td>Fla + Dextrose (Dex)^4</td>
<td>154.6 ± 15.8^a</td>
<td>78.6 ± 1.0^a</td>
</tr>
<tr>
<td>Fla + Dex + Nal</td>
<td>143.3 ± 8.1^b</td>
<td>81.4 ± 1.4^a</td>
</tr>
<tr>
<td>Fla + Dex + SS</td>
<td>84.4 ± 2.6^a</td>
<td>97.9 ± 0.5^a</td>
</tr>
<tr>
<td>Fla + Dex + Nal + SS</td>
<td>142.4 ± 11.2^b</td>
<td>97.9 ± 0.5^a</td>
</tr>
</tbody>
</table>

1Blood glucose was expressed as percentage considering the initial value as 100%
^2Naloxone 5 mg/kg was administered ip 10 min prior to swim stress or acetic acid challenge
^3Flavone 50 mg/kg was administered sc 60 min prior to acetic acid challenge
^4Dextrose 2 g/kg was administered ip 15 min prior to swim stress or acetic acid challenge
^P < 0.001, ^aP < 0.01 compared to vehicle treatment
^bP < 0.001 compared to flavone treatment
^cP < 0.01 compared to swim stress treatment
^dP < 0.01 compared to Fla + SS treatment
^eP < 0.001 compared to Fla + Dex + SS treatment
^fP < 0.01 compared to Dextrose alone value
difference in the blood glucose or serum insulin level or the inhibition of acetic acid induced abdominal constrictions as compared to those of vehicle treated mice (Tables 1 and 2).

**Effect of naloxone on blood glucose level and antinociception in flavone treated mice**—Treatment with naloxone completely antagonized the inhibitory effect of flavone on the abdominal constrictions and this change (flavone vs flavone-naloxone) was statistically significant (P < 0.001). However, the blood glucose level in the flavone treated mice was not significantly modified by naloxone (Table 2).

**Effect of naloxone on blood glucose level and antinociception in swim stress treated mice**—Naloxone treatment partially antagonized the antinociception induced by swim stress. This antagonism was statistically significant (P < 0.01). Naloxone attenuated the swim stress induced hypoglycemia however, this attenuation was not statistically significant (Table 2).

**Effect of naloxone on blood glucose level and antinociception in flavone-swim stress treated mice**—Naloxone treatment totally reversed the flavone induced antinociception (0% inhibition) and partially reversed the swim stress induced antinociception. Conversely, the potentiated antinociceptive response of swim stress by flavone was attenuated by naloxone and this attenuation (flavone-swim stress vs flavone-naloxone-swim stress) was statistically significant (P < 0.01). The hypoglycemia observed in flavone-swim stress treated mice was further enhanced by naloxone administration. However, this mild increase in the hypoglycemic state was not statistically significant. Overall, naloxone did not have any significant influence on the blood glucose level observed in either flavone or swim stress or flavone-swim stress treated mice though significantly antagonized the antinociceptive response to a varying degree in this groups of animals (Table 2).

**Effect of naloxone on blood glucose level and antinociception in flavone-dextrose treated mice**—Though there was a reduction in the blood glucose level induced by naloxone in the flavone-dextrose treated mice, this effect was not statistically significant. Similarly the antinociceptive response was not modified by naloxone in this group of animals. Thus when dextrose was combined with flavone, naloxone failed to antagonise the potentiated antinociception (Table 2).

**Effect of naloxone on blood glucose, serum insulin levels and antinociception in flavone-dextrose-swim stress treated mice**—Naloxone administration reversed the hypoglycemia observed in the flavone-dextrose swim stress treated mice and elicited hyperglycemia and this eliciton was statistically significant (P < 0.001). In contrast, the antinociceptive response in these animals was not modified by naloxone treatment. In other words, naloxone failed to antagonise the antinociceptive response when dextrose was interposed in the flavone-swim stress treated mice, where, surprisingly, in the absence of dextrose treatment, the antinociceptive response was attenuated by naloxone as described earlier. The serum insulin level in flavone-dextrose-swim stress treated mice was 38.5 ± 1.9 μU/mL and after naloxone administration in this group of animals there was no statistically significant difference in the serum insulin level (38.8±1.3 μU/mL) despite a change in the glycemic state from hypo to hyperglycemic (Tables 1 and 2).

**Discussion**

The present new approach has distinct advantages of maintaining intact neuronal function and that the changes are transient but sufficient enough to modify the perception of pain. The inclusion of stress in the paradigm has a distinct advantage as swim stress elicited hypoglycemia as well as antinociception, the parameters with which the present study is concerned. The present approach has a limitation in the sense that the findings cannot be extrapolated to the clinical diabetic chronic state. However, data obtained from the experiments may yield meaningful results reproduced in chronic studies.

The approaches employed in this study are physiological and reflects the true pain threshold in contrast to those recorded in experimental diabetic animals. This is the main advantage of this study.

The flavonoid group of substances is considered safe though milder in eliciting antinociception in contrast to commonly used analgesics like opiates and non-steroid anti-inflammatory agents, which cause severe side effects, they possess anti-ulcer and anti-inflammatory activities besides their antinociceptive property which is a unique combination. Therefore, the possibility of using this type of analgesic in diabetic neuropathy was examined experimentally in this study employing the basic nucleus flavone.

The unaltered glycemic state by the flavone, the hypoglycemia elicited by swim stress, and the hyperglycemia elicited by exogenous administration of dextrose all elicited a significant antinociceptive response. This finding support, the contention that algiesic and glycemic states are dissociated in acute situa-
evidence to this hypothesis. Similarly, the dextrose pretreated antinociception recorded after swim stress in flavone treated animals further confirms that the elevated serum insulin level was uniformly seen whenever antinociceptive activity was recorded irrespective of the glycemic state. It may be appropriate to state that the algesic state is more related to serum insulin level, rather than the blood glucose level. Further experiments on these lines might substantiate this view and propose measurement of serum insulin, a hormonal parameter rather than blood glucose, a metabolic parameter. This might throw more light on the status of diabetes mellitus and appropriate correction. Moreover, it is implied that endogenous insulin may also participate in the control of pain perception physiologically.

The above findings provide sufficient evidences to the hypothesis that no relationship exists between the changes in glycemic and algesic states. At this stage it is worthwhile to ponder the possibility of any intervening factor commonly affecting both the parameters. It has been documented that endogenous opioid peptides modulate physiological pain perception and that they also control the glycemic state through insulin release. Possibly it may be insulin, which is playing a role in such clinical situation. This proposal is supported by the earlier finding that insulin possesses an inherent antinociceptive response. Secondly, the reversal of pain in diabetic neuropathic patients to normal state is achieved through administration of insulin. The findings of the present study with respect to serum insulin level suggest two proposals. A common observation that whenever an antinociceptive response was recorded either through flavone, dextrose or swim stress, the serum insulin level was enhanced (correlation co-efficient value 0.87 indicating a positive correlation) irrespective of the opposite changes recorded in glycemic state. Similarly, whenever potentiated antinociception was recorded through various manoeuvres either a trend or significant further enhancement in serum insulin level was noticed. Besides, the failure by naloxone to antagonise the elevated serum insulin level as well as potentiated antinociceptive response in flavone, dextrose pretreated swim stressed animals further confirms that the elevated serum insulin level was uniformly seen whenever antinociceptive activity was recorded irrespective of the glycemic state. It may be appropriate to state that the algesic state is more related to serum insulin level, rather than the blood glucose level. Further experiments on these lines might substantiate this view and propose measurement of serum insulin, a hormonal parameter rather than blood glucose, a metabolic parameter. This might throw more light on the status of diabetes mellitus and appropriate correction. Moreover, it is implied that endogenous insulin may also participate in the control of pain perception physiologically.

References
9. Schmidt RE, Plurad SB & Roth KA. Effects of chronic experimental streptozotocin-induced diabetes on the nor-


