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A COMPARATIVE STUDY OF ANTIDIABETIC EFFECT OF BROMOCRIPTINE AND METFORMIN IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT: Bromocriptine mesylate quick release formulation has recently been approved by FDA for the treatment of diabetes mellitus. Till now it has been mainly known for its use in Parkinsonism and for suppression of lactation. Present study evaluates and compares the antihyperglycemic effect of the combination of bromocriptine and metformin with either of the two drugs taken alone. For this fasting blood sugar level of all the groups were measured on day 1,7,14 and 21 of treatment. In this study all the three treatment groups was found to have significant ($P < 0.05$) blood glucose lowering effect. Fall in fasting blood was less in bromocriptine treated group as compared to groups treated with metformin and those treated with combination of the two. It is concluded that hypoglycemic effect of combination of metformin and bromocriptine is better than either of the two drugs taken alone. This may be due to potentiation or synergism between metformin and bromocriptine. Further studies are required to prove that bromocriptine has constantly significant hypoglycemic effect with better safety profile than the earlier drugs.

INTRODUCTION: Diabetes mellitus is a leading cause of morbidity and mortality among Indian population. The Diabetes Atlas published by the International Diabetes Federation shows there are currently over 40 million diabetic patients in India¹. The International Diabetes Federation (IDF) reports a projected prevalence of 70 million patients in India by the year 2025 and the World Health Organization (WHO) estimates that India will have 80 million cases of diabetes by 2030². This places India second only to China in terms of number of people living with diabetes³.

By the year 2025, India shall have the maximum number of diabetics in the world making it, the “Diabetic capital of the world”⁴. The prevalence of the disease in adult is 2.4% in rural and 4.0 - 11.6% in urban dwellers⁵.

An ideal oral treatment for diabetes would be a drug that not only controls the glucose level but also delays the development of ESRD and other complications of diabetes. Unfortunately, among the currently available drugs, the choice is very limited. Alloxan monohydrate is known for its selective pancreatic islet beta cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals^{6, 7}. The quick release formulation of bromocriptine mesylate has been approved by FDA in May 2009 for the treatment of type 2 diabetes in adults as an adjunct to diet and exercise to improve glycemic control^{8, 9}.

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The mechanism by which bromocriptine improves hyperglycemia still not very clear. The idea of using bromocriptine for the treatment of type 2 diabetes came while studying the metabolism of migrating birds. It was noted that their hypothalamus have the ability to develop seasonal insulin resistance by controlling their metabolism¹⁰. Reduction in hypothalamic dopaminergic activity and increased noradrenergic activity in the CNS results in increased fat stores and decreased insulin sensitivity resulting in obesity and insulin resistance and glucose intolerance, the pathology most often seen in patient with diabetes mellitus¹¹ (see **figure 1**).

Bromocriptine is a sympatholytic dopamine D2 receptor agonist¹². It is unique in that it doesnot have a specific receptor that mediates its action on glucose and lipid metabolism. Its effects are mediated via resetting of dopaminergic and sympathetic tone within the CNS¹³. Many studies have reported the finding that systemic and

intracerebral bromocriptine administration in insulin-resistant animals decreases the elevated noradrenergic and serotonergic levels in the ventromedial hypothalamus (measured in vivo by microdialysis) with a resultant decline in hepatic glucose production/gluconeogenesis, reduced adipose tissue lipolysis, and improved insulin sensitivity^{14, 15}.

Systemic bromocriptine also inhibits responsiveness of ventromedial hypothalamus to norepinephrine, and conversely, norepinephrine infusion into the ventromedial hypothalamus antagonizes the beneficial effect of bromocriptine on glucose tolerance and insulin sensitivity¹⁶. Consistent with these observations in animals, systemic bromocriptine administration improves glycemic control and dyslipidemia without change in body weight in type 2 diabetic and obese nondiabetic humans¹⁷. The proposed mechanism of action of bromocriptine to improve glucose tolerance is summarized in adjacent figure.

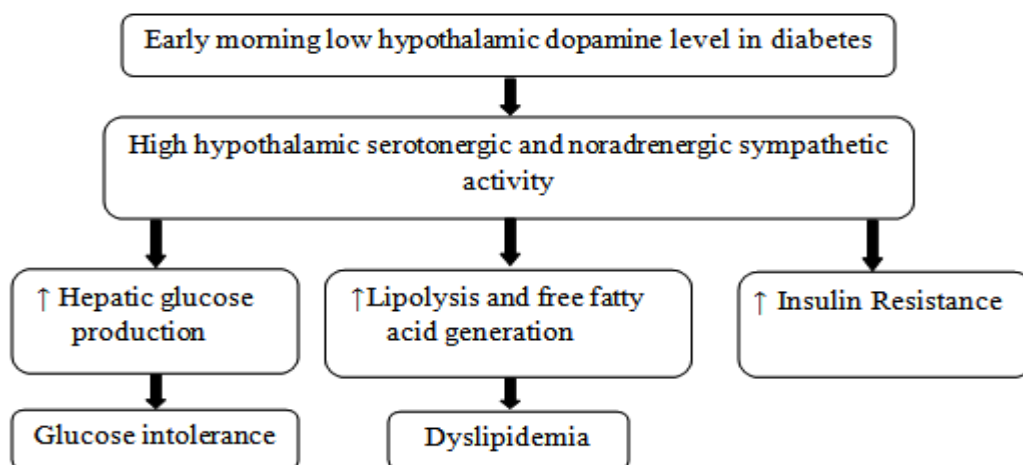


FIGURE 1: PATHOPHYSIOLOGY OF DOPAMINE PATHWAY CONTROLLING BLOOD SUGAR

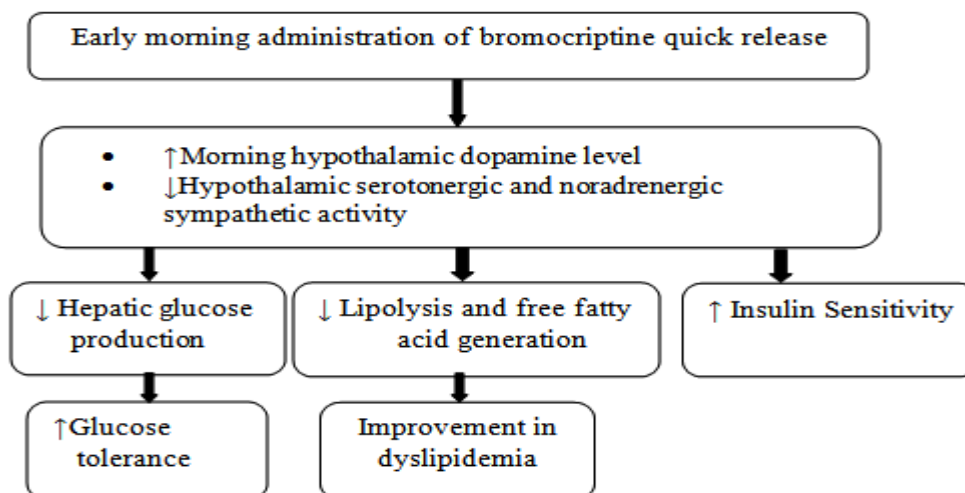


FIGURE 2: SUGGESTED MECHANISM TO IMPROVE GLUCOSE TOLERANCE BY BROMOCRIPTINE

A quick release formulation of Bromocriptine, administered within two hours of awakening, is believed to augment low hypothalamic dopamine level and inhibit excessive sympathetic noradrenergic tone within central nervous system, resulting in decrease in plasma glucose level, decreased hepatic glucose production, decreased lipolysis and lipogenesis (**Figure 2**). These factors improve the glucose tolerance, insulin sensitivity and decrease plasma free fatty acid; triglyceride thus improves glycemic control in diabetic patients¹⁸.

MATERIAL AND METHODS: Healthy male wistar rats weighing between 150-225 grams were taken for the present study. The animals were kept in clean and dry cages, with 12 h: 12 h light-dark cycle at room temperature and humidity. They were acclimatized to the available housing condition and were fed with diet consisting of soaked black gram (Kala Chana) and water was given ad libitum. Arrangements were made to ensure regular cleaning of cages and disposal of excreta and urine. The cages were floored with a layer of saw dust for absorption of urine of rats. This was done because after induction of diabetes by alloxan there was excess of urination. The whole experiment was conducted in accordance with ethical guidelines approved by Institutional Animal Ethics Committee (IAEC) of Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India.

Drugs used;

1. Alloxan monohydrate (10 g) powder – From Sigma Aldrich Chemicals Private Limited (Bangalore).
2. Metformin (Tab. Gluconorm 500 mg) - from Lupin pharmaceuticals.
3. Glucomind (Tab. Bromocriptine mesylate 0.8 mg quick release) - from Lupin pharmaceuticals.

Chemical and reagent kits;

1. Distilled water
2. Dettol and sterilized cotton
3. Normal Saline

4. Glucometer
5. Glucometer strips
6. Insulin syringe
7. Disposable syringe 5 ml
8. Animal Feeding needle (Gavage tube)
9. Sterilized needle and surgical blades

Induction of diabetes mellitus: Male Wistar rats were acclimatized for one week before the initiation of the study. Leaving aside six rats for normal control group, diabetes was induced in overnight fasted rats by single intraperitoneal injection of freshly prepared 1% alloxan monohydrate in sterile normal saline in a dose of 120 mg/kg body weight. After this, they were provided with free access to water and 5% glucose solution overnight to overcome the drug induced hypoglycemia.

The fasting blood glucose level was determined after 72 hours of alloxan injection. The rats having blood glucose levels above 250 mg/dl were used for the study. The diabetic animals were allowed free access to tap water, normal laboratory diet, and were maintained at room temperature in their cages.

Study design: The entire experiment was carried out in “Department of Pharmacology Rajendra Institute of Medical Sciences, Ranchi” after taking permission from the Institutional Animal Ethics Committee (IAEC) of Rajendra Institute of Medical Sciences, Ranchi, and Jharkhand, India.

Total thirty animals were used for the study. First group having six rats were kept nondiabetic. After confirmation of diabetes and stabilization of fasting blood sugar i.e. on 3rd day the diabetic rats were divided into four groups with six rats in each group. Drugs were administered from 4th day and this was considered as day one of the treatment. All the treatments were carried out for a period of 21 days. The fasting blood samples were collected before the induction of diabetes as well as on days 1, 7, 14 and 21 to determine the glucose level by Glucometer.

Selection Criteria for animals:

1. All the animals used for the study were healthy and active in their cage.
2. Animals were male Wistar rats.
3. Weight of the animal used was 150-225 grams.
4. Rats with fasting blood sugar within the range of 250-400 mg/dl were selected for the study.

Rats were given respective treatment orally once daily for 21 days just before feeding in the morning hour at 10-10.30 am. Group A animals were non diabetic and served as normal control and received 0.9% normal saline. Animals with moderate hyperglycemia i.e. serum glucose between 250-400mg/dl were randomly divided into four diabetic groups of six rats in each group. Group B rats served as diabetic control. Group C, which served as positive control, was treated with metformin (9 mg/200g b.w., p.o.). Group D, diabetic rats were treated with bromocriptine (.03 mg/200g b.w., p.o.) and Group E; diabetic rats were treated with combination of bromocriptine (.03 mg/200g b.w., p.o) with metformin (9 mg/200g b.w., p.o.).

The details of groups were as follows;

1. Group A: Normal control
2. Group B: Diabetic control
3. Group C: Diabetes + Metformin
4. Group D: Diabetes + Bromocriptine
5. Group E: Diabetes + Bromocriptine + Metformin

Estimation of fasting blood sugar: For the estimation of fasting blood sugar, the rats were kept deprived of food overnight and were allowed free access to water. Blood samples were collected from the tail of rat and fasting blood glucose was measured with the help of Glucometer.

Statistical analysis: All the data were expressed as mean \pm SD. Statistical analysis of data was carried out by employing ANOVA (one way analysis of

variance) test and Tukey's HSD test was used to compare the effect of drugs on different group. Results were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION:

Effect of alloxan on normal rats: As shown in **table 1**, alloxan treated rats show significant ($P < 0.05$) high value of fasting blood sugar in the entire study duration. The value of FBS in this group was progressively increased due to the diabetes. This also gives the confirmation of successful induction and maintenance of diabetes in the study period.

Effect of drug administration on diabetic rats: Diabetic rats that were treated with different drugs show progressive decrease in FBS value during the study period. In metformin treated group, FBS decreased significantly ($P < 0.05$) in first week and second weeks followed by mild decrease in FBS during third week. In bromocriptine treated group also decrease in FBS was significant ($P < 0.05$) but the decrease in value of FBS after one week was more in metformin treated group as compared to bromocriptine treated group.

Later on decrease in FBS in bromocriptine treated group was more in the second week followed by less significant ($P > 0.05$) change in FBS on third week. Thus maximum effect of bromocriptine was seen on second week and final value at the end of treatment was in higher than normal range. Thus bromocriptine alone was inferior to metformin as a monotherapy in treatment of diabetes. In the fifth group that was treated with combination of metformin and bromocriptine, fall in FBS was more prominent on first week as compared to other two groups treated with metformin and bromocriptine alone. This decrease in FBS was quiet significant during second week reaching almost to normal range.

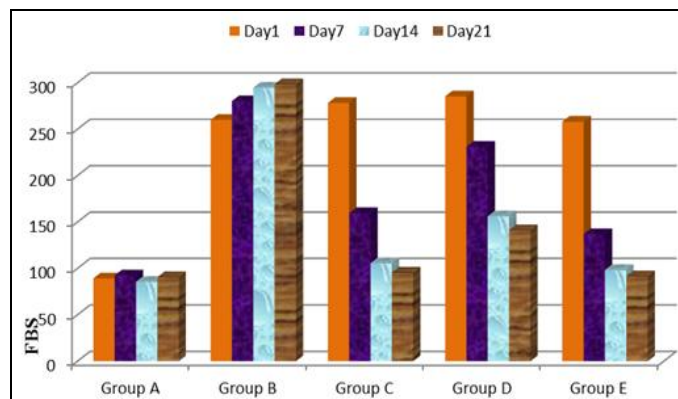
Fall in FBS was mild during third week i.e. FBS was maintained in normal range. Final value of FBS for this group was very near to the FBS in normal control group for the same duration of treatment. Thus this treatment was found to be superior to metformin and bromocriptine monotherapy in treatment of diabetes.

TABLE 1: SEQUENTIAL CHANGES IN FASTING BLOOD SUGAR OVER THE STUDY PERIOD IN ALL STUDY GROUPS

Days	Group A	Group B	Group C	Group D	Group E
Day 1	89.33±1.63	260.16±6.04*	278.33±6.08*	285.4±6.01*	258.16±3.97*
Day 7	92.6±4.08	280.33±2.94*	159.6±5.39*#	231.16±4.8*	137.1±5.07*#
Day14	85.6±3.20	294.83±2.13*	105.5±4.08#	156.16±6.91*#	98.3±5.08#
Day21	90.6±3.77	298.6±7.03*	95.5±4.18#	141.3±5.20*#	91.6±3.38#

Values are expressed in mean ± standard deviation (n=6); *values are statistically significant as compared to group A; #values are statistically significant as compared to group B.

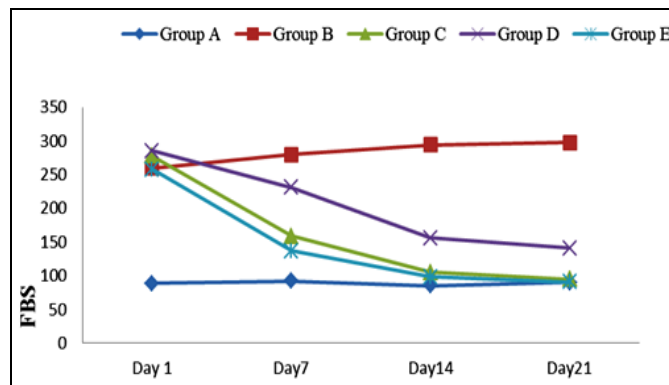
Graph 1 gives the representation of the effect of drug administration on alloxan induced diabetic rats. As shown in the graph, FBS value during the entire study period in normal control group was maintained at a constant level with minor fluctuations and this value was significantly ($P < 0.05$) lower than the diabetic control rats. Diabetic control group show small increment in FBS during 1st, 7th, 14th and 21st day of study. On 7th day of experiment there was fall in FBS values in drug treated group while in untreated group FBS increased progressively.

**GRAPH 1: EFFECT OF DRUG ADMINISTRATION ON ALLOXAN INDUCED DIABETIC RATS**

FBS values taken on 7th day of study show that the fall in FBS was more in Combination treated group and metformin treated group while bromocriptine treated group show moderate fall in FBS.

Also, rate of fall of FBS, (shown by slope of graph in **Graph 2**) was more in combination treated group followed by metformin treated group and it was least in bromocriptine treated group. After 14th day of treatment, FBS in combination treated and metformin treated group comes to near normal values but in bromocriptine treated group though the value of FBS was less than 7th day FBS but it was at higher range. At the end of experiment i.e. on 21st day, FBS in combination treated and metformin treated group was maintained at near

normal value but FBS in bromocriptine treated group remained at higher level as compared to the other two groups.

**GRAPH 2: RATE OF CHANGE OF FASTING BLOOD SUGAR IN ALLOXAN INDUCED DIABETIC RATS**

CONCLUSION: From the above discussion following conclusion can be drawn

1. Bromocriptine has moderate effect in lowering FBS on 7th day and in subsequent week it has more prominent effect on lowering of FBS. Further on third week it doesnot have much effect in lowering of FBS.
2. Metformin in comparison to bromocriptine showed marked decrease in fasting blood sugar on 7th day of the study and decrease in FBS on second week is also very marked. After this FBS comes to a normal range and it maintains this value on 21st day. So it has got faster onset of action as compared to bromocriptine.
3. Combination of Metformin with Bromocriptine has good fasting blood sugar lowering effect on 7th day of the study and on 14th day FBS value comes down to normal value and this does not cause further fall in FBS and this value was maintained at 21st day.
4. Magnitude of fall in this group was higher than the above two groups and normal FBS level was achieved early in this group.

5. Thus, this study concludes that individually both metformin and bromocriptine are effective in controlling hyperglycemia but the combination of metformin with bromocriptine is better in achieving normal fasting blood sugar level than either of the two drugs taken alone.

LIMITATIONS AND SCOPE OF STUDY:

Further studies are required to ascertain the consistency in hypoglycemic effect of bromocriptine. Study taking higher dose of bromocriptine or with increased duration of study can justify its role in achieving proper glycemic control considering slower onset of its hypoglycemic effect in this study.

Study with different group of oral hypoglycemic drugs can be done to assess its value as add-on drugs in the treatment of diabetes and as an adjunct to diet and exercise in controlling initial phase of diabetes mellitus. Also most of the persons suffering from diabetes are obese, so studies for antiobesity effect of bromocriptine can be done as a carry forward study, which can prove its action in improving the lipid profile and cardiovascular outcomes.

In our study, the dose of bromocriptine used was very small as compared to that used in Prolactinoma and Parkinsonism, so considering its safety parameters, study with different higher dosage of bromocriptine can be done as a carry forward study to establish its role in diabetes. This study was done on fasting rats, so further study is needed to see its effect on post prandial blood sugar. There is further scope of doing animal studies involving large number of animals, as well as human clinical trials to evaluate its role in

hyperglycemia, lipid profile and improved cardiovascular outcomes.

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