ORIGINAL ARTICLE

Butea monosperma Reduces Haloperidol and Sulpiride Induced Hyperprolactinemia in Rats

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Abstract:

Background: Prolonged administration of neuroleptic drugs cause disrupted D_2 dopamine receptor which leads to increased prolactin level, causes gyanaecomastia. Presently, dopamine receptor agonist is the choice of treatment for hyperprolactinemia. *Aim and Objectives:* The study aims to determine the anti-hyperprolactinemic effect of methanolic extract of *Butea monosperma* (MEBM) against haloperidol (HPL) and sulpiride (SPD) induced hyperprolactinemia and to correlate with its active constituents. *Material and Methods:* To induce hyperprolactinemia HPL 5 mg/kg for 16 continuous days and SPD 20 mg/kg for 28 continuous days was administred. MEBM 200 mg/kg/day and 400 mg/kg/day were administered for 16 and 28 days respectively half an hour before administration of HPL and SPD. The serum prolactin (PRL) level, dopamine (DA) level and antioxidant status in the rat brain, hematological parameters were measured and histological examination of the anterior pituitary gland, adrenal gland and spleen were done. In addition, antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) were also estimated. *Results:* MEBM decreases serum PRL level and increased DA level in brain significantly. Further, MEBM also restored SOD and CAT status significantly. The inflammatory markers induced by HPL and SPD were suppressed by MEBM. *Discussion:* Neuronal DA inhibition by neuroleptic drugs decreases the release of DA which leads to hyperprolactinemia. MEBM (butrin) may activate DA neurones to ameliorate hyper-prolactinaemia. The dopaminergic, anti-oxidant and anti-inflammatory effect of MEBM may be attributed to its anti-hyperprolactinemic effect. *Conclusion: Butea monosperma* possesses anti-hyperprolactinemic effect which may be attributed to its marker constituent like Butrin.

Keywords: Haloperidol, sulpiride, prolactin, dopamine, catalase, superoxide dismutase

Introduction:

Prolonged administration of neuroleptic drugs cause disrupted D_2 dopamine receptor which leads to increased prolactin level, causes gyanaeco-mastia [1, 2]. Presently, dopamine receptor agonist is the choice of treatment for hyperpro-lactinemia [3]. Cabergoline is the drug of the first choice as it is safe and efficacious. Bromocriptine is the drug of the second choice [2, 4]. There are certain limitations of dopamine agonists. Bromocriptine monotherapy for the treatment of hyperprolactinemia revealed that about 75% patients remained well during a period of 1 year treatment and about 50% patients required a supplement of levodopa (l-dopa) after 2 years of the treatment to attenuate the prolactin level [5, 6]. So, there is a need to find out specific alternative to counter the side effects of antipsychotic drug-induced hyperprolactinemia. *Butea monosperma* alters the hormonal level due to the presence of active constituents like butrin [7]. The present

research work is studied to evaluate the anti-hyperprolactinaemic effect of *Butea monosperma* against haloperidol (HPL) and Sulpiride (SPD) induced hyperprolactinemia.

Material and Methods:

Animals for experiment:

Wistar albino rats were obtained from the animal house of School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Bhubaneswar. All conditions were maintained according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) norms. The wistar albino rats of either sex (weight 120 ± 5g) were selected randomly from animal house. The room temperature was maintained 22 ± 2°C with food and water *ad libitum*. The animals were transferred to the laboratory at least 1h before the start of the experiment. The experiments were performed during day time (08:00-16:00 h). The study was conducted according to CPCSEA guidelines after due approval of the experimental protocol from Institutional Animal Ethics committee, SOA University, (reg. no. 1171/PO/Re/S/08/CPCSEA).

Plant collection:

Healthy disease free, mature fresh Stem of *Butea Monosperma* (Lam.) plants were collected locally from Bilaspur Chhattisgarh, India. The time of collection was in August 2016 during day time.

Plant authentication:

The plants were taxonomically identified by the Dr. P.C. Panda, Principal Scientist of Regional Plant Resource Center (RPRC), Forest and Environment Department, Government of Odisha Bhubaneswar, Odisha (accession field no. PT-03 *B. monosperma*). The authenticated plant was kept in the herbarium file for the record purpose.

Preparation of MEBM extract:

Stem of *B. Monosperma* (Lam.) was shaded-dried at room temperature (27°C) for a week without any contamination. They were then weighed several times until the constant weight achieved. The dried stem part was then ground into a fine powder with the help of grinder and the powder was preserved in an airtight amber container for extraction procedure. The dried powder (250 g) of stem part was extracted with methanol in a soxhlet apparatus at 60–70°C each for 10–12 h consecutively. Extraction was continued till clear solvent was observed in siphon tube. Extracts were concentrated in a water bath at 40°C. Concentrated extract was dried at 40°C in a hot air oven. The percentage yield value was recorded about 14.8 %. The dried extract was stored in sterile amber color in an air tight container in refrigerator until its use in experiment [8].

Preliminary phytochemical screening:

The crude extracts of *B. monosperma* were subjected to different chemical tests for the detection of phytoconstituents such as tannins, alkaloids, saponins, phenolics, flavonoids, glycosides, triterpenoids, steroids, volatile oilsusing standard method [9].

Acute toxicity study:

The acute oral toxicity of *B. monosperma* crude extract was evaluated in albino rats according to the procedures outlined by Organisation for Economic Co-operation and Development (OECD) guideline 420. *B. monosperma* crude extract was administered orally to different groups (each of three animals) in increasing dose levels of 5, 50, 300, and 2000 mg/kg body weight. Animals were observed continuously for 1 hour, then frequently for 24 hours thereafter once daily for 14 days. During that period animals were

observed for gross behavioral and morphological changes.

Drug and treatment:

HPL (HiMedia Laboratories Pvt. Ltd, Mumbai, India) 5 mg/10ml/kg/daily for 16 days and SPD (Unimed Technologies Ltd, Panchmahal, India) 20 mg/10ml/kg/daily for 28 days were administered intraperitoneally (i.p.). Plant extracts were administered p.o. daily 30 minutes before administration of HPL/SPD [10, 11].

Serum collection and storage:

Blood samples of control and treated animals for hormone assay were collected by cardiac puncture. Serum samples were separated by centrifugation, frozen and stored at -4^{0}C until use. The concentration of PRL hormone was determined by enzyme immune assay method using rat prolactin kit [12].

Pharmacological Evaluation of Hyperprolactinemia:

The Methanolic Extract of *Butea monosperma* stem (MEBM) was tested against Haloperidol (HPL 5 mg/kg) and Sulpiride (SPD 20 mg/kg) induced hyperprolactinemia. The treatment protocol was designed as follows:

Haloperidol + MEBM:

Animal were divided into four (4) groups of six (6) animals each. In each group three male and three female animals were used. First group received (Saline 2 ml/kg/day), second group received HPL 5 mg/kg/day, third group received HPL 5 mg/kg/day + MEBM 200 mg/kg/daily and fourth group received HPL 5 mg/kg/day + MEBM 400 mg/kg/daily, The drugs were administred once daily (8 to 10 am) for 16 continuous days [10]. On the 17th day all animals were sacrificed and blood was drawn for measurement of PRL.

Sulpiride + MEBM:

Similarly, animals were divided into four (4) groups of six (6) animals each. First group received (saline 2 ml/kg/day), second group received SPD 20 mg/kg/day, third group received SPD 20 mg/kg/day + MEBM 200 mg/kg/daily and fourth group received SPD 20 mg/kg/day + MEBM 400 mg/kg/daily. The drugs were intraperitoneally administered once daily (8 to 10 am) for 28 continuous days [11]. On the 29th day, all animals were sacrificed and blood was collected for measurement of PRL.

Dopamine Estimation:

The entire brain of a rat was collected and weighed. Homogenization of brain tissues was performed for about one minute in HCl-butanol (1:10) mixture. These homogenized samples were then subjected to centrifugation at 3000 rotation per minute (rpm) for about 10 minutes. One ml of aliquot supernatant phase was removed followed by its addition to centrifuge tube comprising 2.5ml hexane and 0.3 ml of 0.1M HCl. The dopamine assay was then performed by taking 0.2ml of the aqueous phase. The temperature was maintained at 0^{0}C. Then, 0.05 ml 0.4 M HCl, and 0.1 ml of sodium acetate buffer of pH 6.9 were added to 0.5 ml of an aqueous phase. This was followed by addition of 0.1 ml iodine solution in 0.1M ethanol for oxidation. This reaction was then interrupted for 2 minutes by addition of 0.1ml of sodium sulfate solution. After 1.5 minutes, 0.1ml of acetic acid was added. This solution was then subjected to heating for duration of 6 minutes. Then the sample was cooled. When the temperature of the sample was dropped down to room temperature, excitation and emission spectra was performed with spectrofluorimeter at 330-375 nm [13].

Assessment of Antioxidant status:

Assay of Superoxide dismutase (SOD):

The whole brain was homogenized by addition of 1mL 0.05M sodium phosphate buffer and pH=7.0 was maintained during the process of homogenization. The homogenate was centrifuged at 10000 rounds per minute (rpm) for 15 minutes at 40C. Thereafter supernatant was collected and stored at -80^{0}C to estimate SOD content. In brief 0.5 mL of EDTA (1mM) and 1.5 mL of Tris buffer (0.05M) mixed together and considered as a blank solution. In case of control 1 mL of Pyrogallol (0.2mM) was added to the identical preparation. For test preparation, 50 µL of serum or brain homogenate was added to the similar blank preparation. Then variation in absorbance was observed at 420 nm by using JASCO (V-630) UV spectrophotometer against reagent blank and measure status/concentration of oxidative stress in rat brain. The data are expressed as µ/mg protein [14, 15].

Assay of Catalase (CAT):

For the estimation of CAT activity, the whole brain tissue was homogenized similar to SOD assay. Thereafter, about 20µL tissue supernatant was added to the 980µl of the assay mixture consisting of 900 µl of 10m mol/L of H_2O_2, 50µl of Tris HCl buffer (pH-8) and 30µl of distilled water. Then variation in absorbance was measured at 240 nm. The results were expressed as µ/mg protein [16].

Haematological Studies:

There is a correlation between central nervous system (CNS) inflammations with peripheral inflammation which made a coupling bridge. So to assess peripheral inflammation hematological study were conducted. Blood were taken from the apex of the left ventricle of the heart with a syringe and collected in tubes containing EDTA as an anticoagulant and the blood sample analysis was carried out by hematological (autoanalyzer) where red blood cell count (RBC), total leukocyte count (TLC), hemoglobin (Hb), packed cell volume (PCV), platelet, mean corpuscular volume (MCV) were measured. The weight of spleen was measured [17, 18].

Isolation of butrin:

The crude extract was dissolved in hot methanol and kept for cooling at room temperature. Thereafter few ml of diethyl ether was added into the obtained solution. The entire solution was then stored in a refrigerator for the period of three days for the purpose of recrystallization and then the white solid was obtained after the recrystallization process. Methanol was then added to the white solid to get colorless needle-shaped Butrin [19, 20].

High-Performance Liquid Chromatography – Mass Spectrometry (LC-MS):

LC-MS analysis was carried out with the help of Agilent Technologies (6545 Q-TOF LC/MS) instrument at the Central Instrumentation Facility (CIF), Birla Institute of Technology and Science (BITS), Pilani.

Histopathological examination of pituitary gland, adrenal gland and spleen:

Animals from each group were sacrificed 24 hr after last treatment (on 17th and 29th days) following ethical procedure. For histopathological examination brain, adrenal gland and spleen were separated and stored in 10% formalin solution. Brain and spleen samples embedded in paraffin wax was used for a serial section at 5 µm and stained with Hematoxylin-Eosin (HE) and mounted on a glass slide for microscopic evaluation [21].

Statistical analysis:

The data obtained by the various parameters in the study were presented as Mean ± Standard Error of Mean (SEM). One-way Analysis of Variance (ANOVA) followed by post hoc Tukey's t-test was applied for statistical analysis.

Results:

Anti-hyperprolactinemic effect:

The control group normal serum PRL level was 10.95±0.45 ng/mL. After prolonged administration of HPL (5 mg/kg/day for 16 days) and SPD (20 mg/kg/day for 28 days) serum PRL level was significantly ($p<0.05$) increased to 25.10± 0.35ng/mL and 19.86±0.37 ng/mL respectively. MEBM 200 mg/kg/day and 400 mg/kg/day significantly ($p<0.05$) decreased prolactin level to 15.60±1.35 ng/mL and 14.74±0.57 ng/mL respectively in HPL treated rats. In addition, MEBM 200 mg/kg/day and 400 mg/kg/day significantly ($p<0.05$) decreased serum PRL level to 14.14±0.79 ng/mL and to 13.78±1.08 ng/mL respectively in SPD induced hyperprolactinemic rats (Fig.1).

Dopaminergic action:

Dopaminergic effect of MEBM was also evaluated against HPL induced hyperprolactinemia. In our study, we have observed that DA level was 38.24±0.04 units/g in control rat brain. Prolonged administration of HPL significantly ($p<0.05$) decreased dopamine level to 11.52±0.00 units/gand administration of SPD significantly decreaseddopamine level to 14.14±0.03 units/g. Furthermore, administration of BM 200 mg/kg/day and 400 mg/kg/day showed significant ($p<0.05$) increase in DA level to 33.17±0.72units/g and 33.29±0.53 units/g respectively in HPL induced hyperprolactinemic rats and to 32.00±0.14 units/g and 32.56±0.15 units/g respectively in SPD induced hyperprolactinemic rats (Fig. 2).

Antioxidant status of SOD and CAT:

The study revealed that prolonged administration of HPL 5 mg/kg/day for 16 continuous days and SPD 20 mg/kg/day for 28 continuous days decreased SOD and CAT levels than the toxicant control group of animals (Table 1). The levels were significantly protected by MEBM 200 mg/kg/day and 400 mg/kg/day.

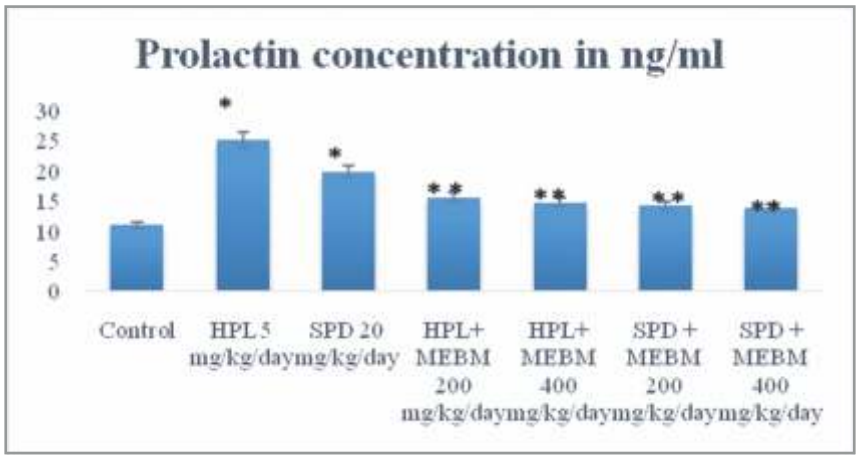

Fig. 1: Effect of Haloperidol (HPL), Sulpiride (SPD) and MEBM on Blood Serum PRL Level in Rats.

*Data expressed as mean ± SEM, n= 6, One way ANOVA followed by post hoc Tukey's test, * $p<0.05$ (control Vs HPL, SPD), ** $p<0.05$ (HPL, SPD vs test).*

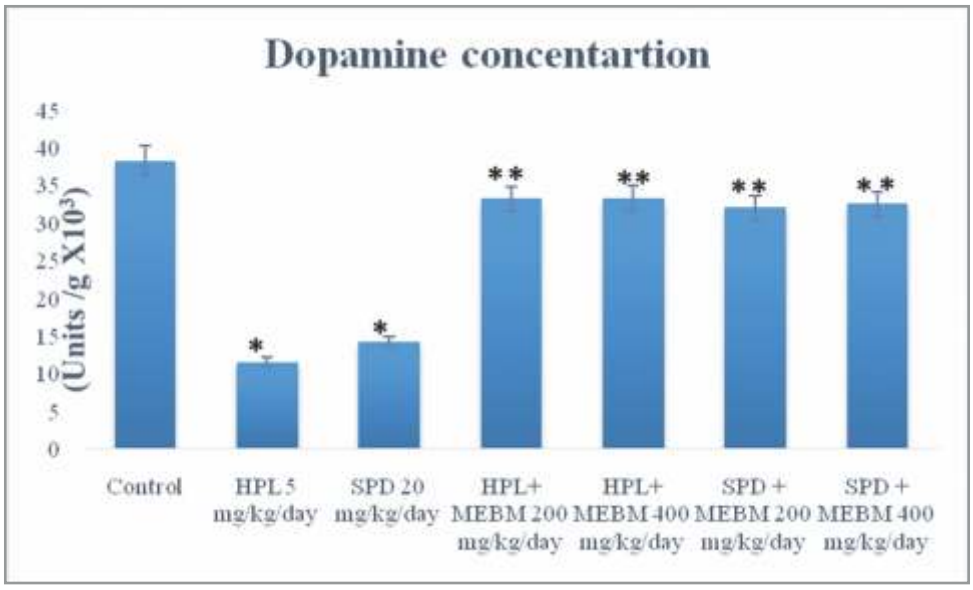

Fig. 2: Effect of Haloperidol (HPL), Sulpiride (SPD) and MEBM on Brain Neurotransmitter Levels (Dopamine) in Rats.

*Data expressed as mean ± SEM, n= 6, One way ANOVA followed by post hoc Tukey's test, * p<0.05 (control Vs HPL, SPD), ** p<0.05 (HPL, SPD vs test).*

Table 1: Effect of MEBM on Antioxidant Status in Haloperidol and Sulpiride Treated Rats

Groups	Dose	SOD (µ/mg protein) mean ± SEM	CAT (µ/mg protein) mean ± SEM
Control	Saline 2 ml/kg/day, i.p	11.25±0.00	2.06±0.00
Haloperidol	5 mg/kg/day	5.27±0.00*	0.68±0.00*
Sulpiride	20 mg/kg/day	6.32±0.00*	0.85±0.00*
MEBM + HPL	200 mg/kg/day+ 5 mg	8.54±0.00#	0.94±0.00**
MEBM + HPL	400 mg/kg/day+ 5 mg	9.86±0.00$	1.15±0.00**
MEBM + SPD	200 mg/kg/day+ 20 mg	7.93±0.00#	0.98±0.00**
MEBM + SPD	400 mg/kg/day+ 20 mg	8.96±0.00$	1.22±0.00**

Data expressed as mean ± SEM, n= 6, One way ANOVA followed by post hoc Tukey's test, * p<0.05 (control Vs SPD), ** p<0.05 (SPD vs test), $ p<0.05 (test 200 vs test 400).

Hematological evaluation:

HPL and SPD treated groups showed significant decrease in red blood cells (RBC) and hemoglobin (Hb) count whereas MEBM 200 mg/kg/day and 400 mg/kg/day showed significant increase in RBCand Hb count as compared to HPL and SPD treated groups. Further, HPL and SPD groups showed a significant increase in TLC count, PCV count, platelet count and MCV (Table 2). On the other hand, MEBM 200 mg/kg/day and MEBM 400 mg/kg/day showed a significant drop in TLC, PCV, platelet, and MCV.

Phytochemistry:

Flavonoids are found to be present in MEBM after phytochemical screening. Thin layer chromatography showed spots with retention factor having (R_f) values of 0.21, 0.33, 0.39, 0.46, 0.69, 0.79, 0.90, and 0.96. LC/MS of crude extract was also done and then isolation was performed. The comparative LC/MS study of both crude extract and Butrin is depicted in (Fig. 3 and Fig. 4).

Table 2: Effect of MEBM on Hematological Parameters in Haloperidol and Sulpiride Treated Rats (n=6)

Treatment (dose mg/kg/day)	Red Blood Cell Count x10^3cell/µL	Total leukocyte Count x10^3cell/µL	Hemoglobin (g Hb/DL)	PCV (%)	Platelet (× 10^9 per litter)	MCV (liters/cell)	Spleen weight in grams
Control (normal saline)	7.32±0.00	3.71±0.00	14.38±0.00	43.42±0.00	1.40±0.00	59.37±0.00	0.77±0.00
Haloperidol 5 mg/kg/day	6.17±0.00 *	6.94±0.00*	12.60±0.00*	44.84±0.00*	2.11±0.00*	61.77±0.00*	0.88±0.00*
Sulpiride 20 mg/kg/day	6.22±0.00 *	6.83±0.00*	12.67±0.00*	44.70±0.00*	2.05±0.00*	61.61±0.00*	0.87±0.00*
MEBM 200 mg/kg/day+ HPL 5 mg	6.81±0.00**	5.24±0.00**	13.27±0.00**	44.25±0.00**	1.73±0.01**	60.67±0.00**	0.831±0.00**
MEBM 400 mg/kg/day+ HPL 5 mg	6.99±0.00**	4.93±0.00**	13.74±0.00**	43.88±0.00**	1.53±0.01**	59.90±0.00**	0.795±0.00**
MEBM 200 mg/kg/day+ SPD 20 mg	6.80±0.00**	5.47±0.00 **	13.21±0.00**	44.39±0.00	1.78±0.01**	59.97±0.00**	0.826±0.00**
MEBM 400 mg/kg/day+ SPD 20 mg	7.03±0.00**	4.76±0.00**	13.85±0.00**	43.96±0.00**	1.71±0.01 **	59.55±0.00**	0.774±0.00**

*All the values are mean ± SEM of six individual observations (one way ANOVA followed by Tukey's-test). * Indicates significance from control at p<0.05 level.*

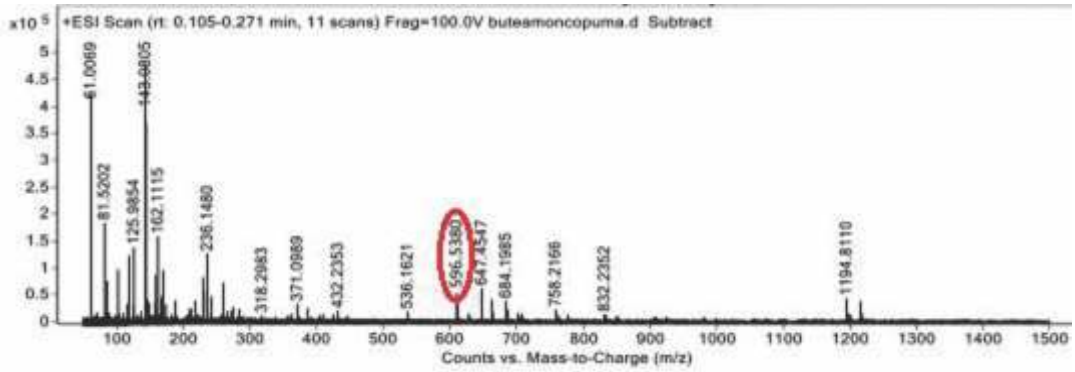

Fig. 3: LC-MS analysis of MEBM

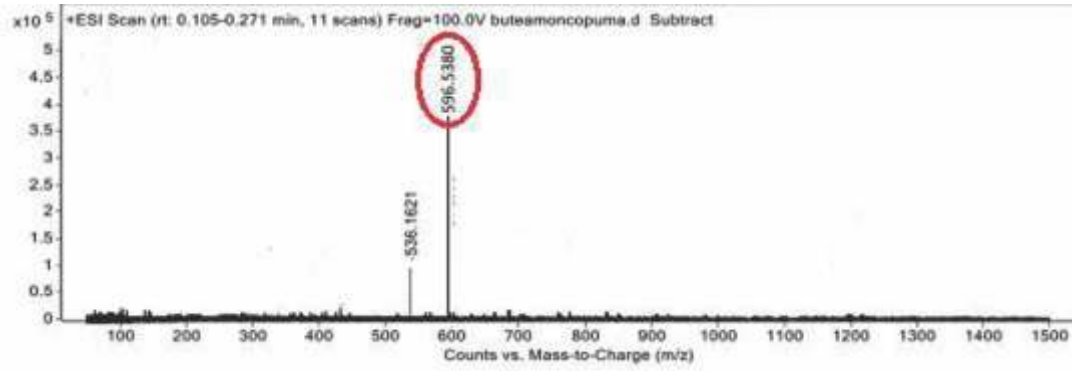

Fig. 4: LC-MS of isolated compound from MEBM (Butrin)

Histopathological examination:

Pituitary gland:

Section of pituitary tissue showed HPL (16 days) and SPD (28 days) treated rats have abnormal brain cytoarchitecture. We observed that in control as well as MEBM treated groups hydrophilic cells were found normal, whereas SPD 20 mg/kg/day treated group shows hydrophilic changes in the cells, which were reversible and haloperidol 5 mg/kg/day treated group shows atrophy of the cells which were irreversible (Fig. 5). Both HPL and SPD treated group showed dilated capillary, injured neuronal fibres, irregular acidophil, and basophils cells, and reversible hypertrophic changes in the anterior pituitary gland.

Adrenal gland:

The adrenal gland sections from control, HPL (5 mg/kg/day) for 16 days, SPD (20 mg/kg/day) for 28 days, MEBM 200 mg/kg/day group were found to have the same cytoarchitecture profile with well-developed cortex and medulla (Fig. 6). Moreover, even higher doses of MEBM 400 mg/kg/day have no influence on the adrenal gland cytoarchitecture.

Spleen:

Control group showed no irregularities. It also showed loosely packed Red and White Pulp (RP & WP). The red pulp consists of Sinusoid Spaces (SS), Lymphocytes (LYM) and red blood cells. The white pulps are with a darker staining mantle zone and a lighter staining germinal zone, collapsed

interlobular matrix, a higher number of collapsed melano-macrophages indicating infection. So, the spleen was degraded and damaged with a muddy appearance in HPL and SPD treated groups. MEBM 400 mg/kg/day treated groups observed healthy with brownish reddish appearance and also showed moderate to marked increase in the proportion of white pulp (Fig. 7). Increased white pulp and red pulp ratio specify activation of an immune response. Therefore, MEBM may protect the spleen from HPL and SPD induced infection.

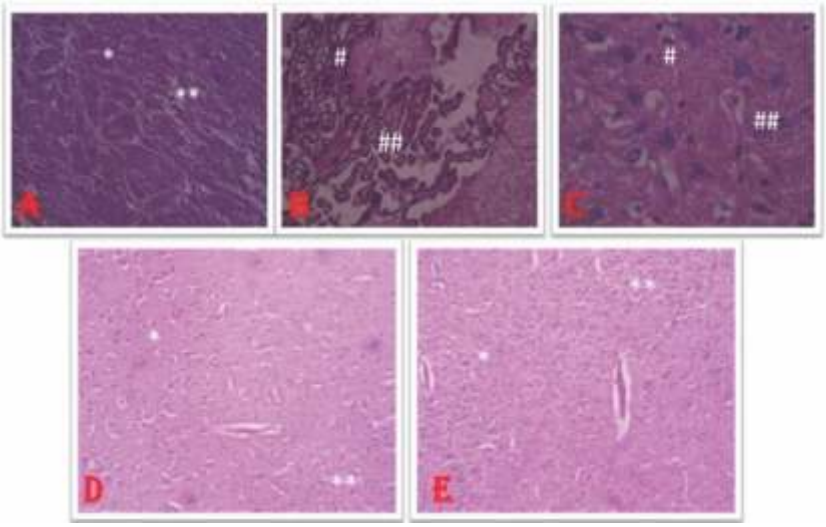

Fig. 5: Histopathology of Anterior Pituitary Gland. A signifies for control, B signifies for haloperidol 5 mg/kg/day, C signifies sulpiride 20 mg/kg/day, D signifies MEBM 200 mg/kg/day and E signifies MEBM 400 mg/kg/day (HE, 100x).

** Indicates normal capillary, neuronal fibres and regular acidophil cell. ** Indicates basophils cell. # Indicates dilated capillary, injured neuronal fibres, irregular acidophil cell. ## Indicates minor basophils cells.*

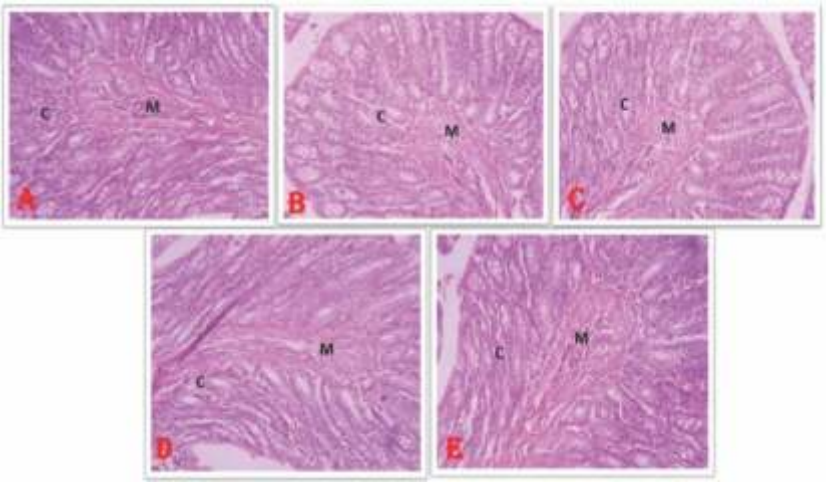

Fig. 6: Histopathology of Adrenal Gland. A stands for Control, B stands for HPL (5 mg/kg/day) for 16 days, C stands for SPD (20 mg/kg/day) for 28 days, D stands for BM (200 mg/kg/day) and E stands for BM (400 mg/kg/day) Medulla (M) and Cortex (C) (HE, 100x).

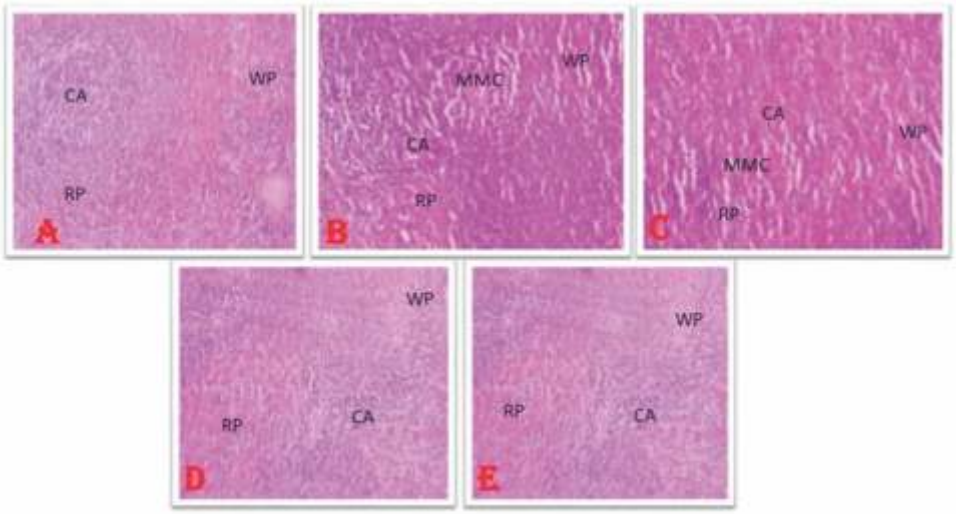

Fig. 7: Histopathology of Spleen. A stands for Control, B stands for HPL (5 mg/kg/day) for 16 days, C stands for SPD (20 mg/kg/day) for 28 days, D stands for BM (200 mg/kg/day) and E stands for BM (400 mg/kg/day) (HE, 100x). Red pulp (RP), White pulp (WP), Central artery (CA) and Melano macrophage centre (MMC).

Discussion:

Antipsychotics, which can also be referred to as neuroleptics, are the class of compounds which have the ability to bind with the dopamine receptors and inhibit dopamine receptors [22], but that leads to enhancement of PRL level (hyperprolactinemia) which limits there utility. So, here administration of HPL 5 mg/kg/day continuously for 16 days and SPD 20 mg/kg/day continuously for 28 days significantly increased serum PRL level which justified our prior work [10, 11]. MEBM significantly reduced hyperprolactinemia in both the models. However, no dose dependency was seen. The result also showed that flavonoids are present in MEBM with Butrin as a marker compound. R_f at 0.33 and 0.46 in TLC confirm the presence of butrin in MEBM. LC/MS of crude extract as well as isolated compound shows 596.5380 molecular weight which confirms that MEBM contains Butrin [20]. Neuronal DA inhibition by neuroleptic drugs decreases the release of DA which leads to hyperprolactinemia [23].

MEBM (200 mg/kg & 400 mg/kg) showed significant increase in the dopamine level in HPL (5mg/kg) and SPD (20 mg/kg) treated rats. The increase in DA concentration could be due to the active component like Butrin. Butrin present in MEBM acts as DA agonist and inhibit the release of prolactin as well as Prolactin-inhibiting Factors, (PIF) and Prolactin-releasing Factor (PRF) such as DA [24, 25]. This effect can also be mediated by several stimulatory pathways which regulate opiates, serotonin and cocaine reuptake [26]. Henceforth, the PRL reducing the effect of Butrin against HPL and SPD induced hyperprolactinemia may be due to the action of active components on the lactotropic cells of the pituitary gland. However, this effect may also be possible due to modification in other pathways [27, 28].

Butrin has a great impact on Adenosine receptors (A1 & A2a). Adenosine A2A receptors are concentrated in the dopamine-rich areas of the brain activate adenylyl cyclase and voltage-sensitive Ca^{2+}-channels involved in motor function [29, 30]. So, it may be considered that A1 & A2a are the target sites for Butrin. So, there may have some other factors contributing to anti-hyperprolactinemic effect besides dopaminergic action. Due to the elevation of PRL level there is decreased DA level which leads to the activation of Reactive Oxygen Species (ROS) and free radicals thereby causing oxidative stress [31]. Our study is in agreement with this as administration of HPL and SPD decreases SOD and CAT level. Free radicals are highly reactive chemicals generated during metabolic processes. The excess amount of free radicals can lead to free radical-mediated neurotoxicity in schizophrenia patients [32]. So, MEBM with antioxidant effects ameliorates neuroprotection in brain of HPL and SPD induced hyperprolactinemic rats [33, 34]. Certain neuroleptic drugs bring immense alteration in hemopoietic cells leading to hematological toxicity [35].These variations could either be dose-dependent [36]. Our experimental data also suggested a decrease in RBC, Hb and increase in TLC, PCV, platelet, MCV, spleen weight in HPL and SPD treated groups which may be attributed to spleen damage or infection or inflammation [37].

So MEBM may possess remarkable neuro-antiinflammatory property in HPL and SPD induced hyperprolactinemic rats. In addition, histological observation of pituitary gland and spleen narrates organ level toxicity produced by neuroleptic drugs but, no effect was seen on the adrenal gland. So MEBM may decrease inflammation in both pituitary gland and spleen. This anti-neuroinflammatory effect may be attributed to the presence of butrin in MEBM. Butrin activates p38 (MAPK/hog) pathway resulting in hemo oxygenase (HO)-1, a potent inhibitor of adipose hypertrophy, inhibits ROS [38, 39] and downregulate the reversible hypertrophy in anterior pituitary gland to show anti-neuroinflammatory (neuroprotective) effect which justifies therapeutic utility of Butrin present in MEBM.

Conclusion:

HPL and SPD induced hyperprolactinemia are associated with a decrease in DA concentration in the brain leading to neuroinflammation as well as alteration of WBC, TLC, Hb, MCV etc, and causes spleen infection. MEBM at 400 mg/kg/day significantly increased DA concentration resulting in decreased serum PRL level. Moreover, there is a maintenance of hematological and histological parameters which indicates inhibition of peripheral as well as neuronal inflammation. Protection against neuroinflammation may be attributed to the use of MEBM or Butrin in drug-induced hyperprolactinemia. Further studies are needed to validate the efficacy of *Butea monosperma* in psychiatric patients.

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