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PHARMACOLOGICAL EVALUATION OF ANTIDEPRESSANT POTENTIAL OF KAEMPFEROL IN ALLOXAN INDUCED DIABETIC RAT

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ABSTRACT

This study aims to evaluate the pharmacological effects of Kaempferol on alloxan-induced diabetic rats. A total of 36 male Sprague Dawley rats will be used, divided into six groups: normal control, diabetes control, and four treatment groups receiving Kaempferol at different doses (25, 50, and 100 mg/kg) or Fluoxetine (10 mg/kg) for 6 weeks. Parameters to be evaluated include body weight, food and water intake, urine output, fasting blood glucose level, and behavioral tests such as elevated plus maze and forced swim test. Ex-vivo parameters including oxidative stress markers and brain GABA levels will also be assessed. The study aims to determine the antidepressant activity of Kaempferol in diabetic rats and provide insights into its potential therapeutic applications.

KEYWORDS: Kaempferol, Alloxan, Diabetes, Fluoxetine.

I. INTRODUCTION

Diabetes is widely recognized as one of the leading causes of death and disability worldwide. The prevalence of diabetes will rise from 6% to over 10% in the next decade. In 2000, the World Health Organization (WHO) recorded a total of 171 million people for all age groups

worldwide (2.8% of the global population) who have diabetes, and the numbers are expected to rise to 366 million (4.4% of the global population) by 2030.[1]

Diabetes mellitus is a disease/group of syndromes characterized by chronic hyperglycemia as a result of either lack of insulin or resistance to its action; hence there is altered.[2] All forms of Diabetes Mellitus are due to either a decrease in the circulating concentration of insulin (insulin deficiency seen in type 1) or a decrease in the response of peripheral tissues to insulin (insulin resistance seen in type2). These abnormalities are what lead to alterations in metabolism of carbohydrates, lipids, ketones and amino acids, which are the features of hyperglycemia. Thus, in the diabetics with either insulin resistance or deficiency, there is increased hepatic glucose production, decreased peripheral glucose uptake and decreased production of glycogen from glucose in the liver.[3]

PATHOPHYSIOLOGY

Insulin facilitates glycogen synthesis from glucose in liver, muscle by stimulating the enzyme, glycogen synthetase. Reduction in insulin release increases glucose level which facilitates reverse conversion of glycogen to glucose. This action is controlled by another hormone, glucagon which acts contrary to insulin. Under constraint, glucose (from glycogen), re-enters the blood when the insulin level is low. When insulin level is high there is an increase in cell growth and duplication, protein synthesis and fat storage. Insulin primarily converts bidirectional processes of metabolism (catabolic to anabolic direction and vice versa). Insulin insufficiency triggers ketosis (fat burning metabolic phase) making the cells poorly respond, leading to insulin insensitivity or Resistance. In case of defective insulin levels, glucose will be neither utilized by the body cells nor stored in liver and muscles. This results in persistent high blood glucose, poor protein synthesis and metabolic derangements like acidosis.

II. MATERIAL AND METHODS

1. Animals

Sprague Dawley rats weighing 180-200 gm. were purchased from National Institute of Biosciences, Pune. The animals were housed in polypropylene cages and maintained under the environmental condition of temperature 25±1 ºC and relative humidity of 45-55 % under 12h light: 12 dark cycles. The animals had free access to food pellets (Nav Maharashtra Chakan oil mills Ltd., Pune) and water ad libitum. The Institutional Animal Ethics Committee (IAEC) number SWGP/LSDP/Form B-3/2024 approved all the experimental

protocols under the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). The protocol approval number is 2168/PO/Re/S/22/CPCSEA.

2. **Chemicals:** Kaempferol, Alloxan, Ether, Acetic acid, Trichloro acetic acid, Potassium dihydrogen Phosphate, Potassium hydroxide, Potassium chloride.
3. **Instruments used:** Glucometer, Animal weighing balance, electronic balance, Spectrofluorometer, UV Spectrophotometer, and Elevated Plus maze apparatus.
4. **Preparation of drug solutions, storage, volume, route of administration:** The study involved preparing drug solutions of kaempferol and fluoxetine, which were stored in airtight amber-colored bottles at room temperature and in a refrigerator, respectively. The volume of drug administration was calculated based on the body weight of animals, and the drugs were administered orally (p.o.).
5. **Experimental design:** The study involved 6 groups of rats, each with 6 rats. The groups were:

i. **Normal group:** (received only vehicle, distilled water)
ii. **Diabetes control group:** (received alloxan and vehicle)
iii. **Fluoxetine-treated group:** (received alloxan and fluoxetine at 10 mg/kg)
iv. **Kaempferol-treated group:** (received alloxan and kaempferol at 25, 50, or 100 mg/kg)

6. **Treatment with Kaempferol and fluoxetine:** Kaempferol and fluoxetine were administered orally from the day of confirmation to 42 days. The observations were recorded on days 0, 14, 28, and 42, and doses were administered immediately afterward.
7. **Parameters:** In-vivo parameters: body weight, urinary output, plasma glucose levels, elevated plus maze test, tail suspension test, and forced swimming test. Ex-vivo parameters: brain oxidative stress (lipid peroxidation, superoxide dismutase, reduced glutathione, nitric oxide, and total protein), brain monoamine levels.

8. METHODOLOGY

1. Induction of diabetes

- **Preparation of alloxan**
- For each experiment, aliquots of alloxan monohydrate from the same batch were pre-weighed into plastic microfuge tubes, then wrapped in aluminum foil (to protect against light sensitivity) and stored at -20°C with desiccant until use. Alloxan monohydrate dissolved in 0.9% w/v cold normal saline, and the unused contents were discarded after each experiment.
- All the required parameters were carried out in all selected rats before diabetes induction.

- All animals fasted for 16 hours before alloxan injection.
- Diabetes was induced by a single injection of alloxan (150 mg/kg) according to the body weight of animals.
- 10% sucrose solution was provided after four hours of alloxan injection to avoid hypoglycemic shocks.
- Diabetes was confirmed after 48 hours of injection by glucometer.
- Glucose level above 250 mg/dl was considered for the study.
- Oral treatment with kaempferol (25, 50 and 100 mg/kg) or Fluoxetine (10 mg/kg) continued for 42 days.
- On the 42nd day, animals were sacrificed for ex-vivo parameters.

3. Treatment of kaempferol and fluoxetine

Kaempferol doses (25, 50, and 100 mg/kg) and fluoxetine (10 mg/kg) with different calculated doses based on the animal's body weight were administered per oral from a day of confirmation to 42 days.

The observations were recorded on days 0, 14, 28, and 42 in the morning, and doses were administrated immediately afterward.

2. Parameter for assessment of the effect of kaempferol on alloxan-induced diabetic depression in rats

1. In-vivo parameters

1. Body weight, food intake, water intake, and urinary output

- Rats were weighed daily using animal weighing balance.
- Food intake, water intake, and urinary output were determined by using a metabolic cage.

2. Blood parameter

- The plasma glucose levels were determined daily by using a glucometer.

3. Elevated plus maze test

- The maze is made of plywood and consists of two open arms 30 X 5 cm and two enclosed arms 30 X 5 X 20 cm. The arms extend from a central 5 X 5cm platform. The maze is dark brown and mounted to the wooded base, raising at 30 cm above the flooring in a dark room. The light of 40W is illuminated above the maze.

- Before starting the experiment, the rats were handled daily to reduce stress. Two hours after the oral administration of the test drugs, the animal was placed in the center of the maze, facing one of the enclosed arms. After that, the number of entries and time spent in the open and closed arms were recorded during the next 5 min. The following parameters were measured: an arm entry being defined when all four paws are in the arm.
- Number of open arm entries
- Number of closed arm entries
- Time spent in open arm
- Time spent in closed arm
- Time spent in a central square
- At the end of each trial, the apparatus was wiped clean to eliminate any olfactory clues, which might modify the behavior of the next animal. The procedure was conducted preferably in a sound-attenuated room, with observations made from an adjacent room.

4. Tail suspension test

- In this TST, rats were individually suspended by the tail from an aluminium hook raised 20 cm above the floor using adhesive tape placed 2 cm from the tip of the tail. The rats were positioned such that the base of their tail was aligned with the horizontal plane.
- Typically, rats demonstrated escape-oriented behavior interspersed with successively longer bouts of immobility. Test sessions lasted for 6 min, and they were videotaped and subsequently scored by a trained observer.

5. Forced swimming test

- The FST apparatus was a transparent Plexiglas cylinder (20 cm diameter, 60.5 cm height). The cylinders were filled with 25 ± 1°C water to a 44.5 cm depth to prevent animals' tails from touching the bottom of the tank. The animals were placed in the cylinders for 5 min. Animals were towel-dried after each exposure and returned to preheated home cages.
- Duration of immobility was measured, i.e., immobility is when the rat performs the minimum movement necessary to stay afloat.

2. Ex-vivo parameters

1. Determination of brain oxidative stress

- All animals were sacrificed at the end of the study, i.e., 42nd day. The brain was immediately isolated.

- Tissue homogenate was prepared with 0.1M Tris-HCl buffer (pH 7.4), and supernatant of homogenate was employed to estimate lipid peroxidation (MDA content), superoxide dismutase (SOD), reduced glutathione (GSH), nitric oxide (NO), and total protein.

1. Determination of Lipid Peroxidation (MDA content)

- It was estimated using the method described by Slater and Sawyer (1971). 2.0 ml of the tissue homogenate (supernatant) was added to 2.0 ml of freshly prepared 10% w/v trichloroacetic acid (TCA), and the mixture was allowed to stand in an ice bath for 15 minutes. After 15 minutes, the precipitate was separated by centrifugation, and 2.0 ml of the clear supernatant solution was mixed with 2.0 ml of freshly prepared thiobarbituric acid (TBA).
- The resulting solution was heated in a boiling water bath for 10 minutes. It was then immediately cooled in an ice bath for 5 minutes. The color developed was measured at 532 nm against the reagent blank. Different concentrations (0-23nM) of standard malondialdehyde were taken and processed for the standard graph.
- The values were expressed as nM of MDA/mg protein.

2. Determination of Superoxide Dismutase (SOD)

- Superoxide dismutase was estimated using the method developed by Misera and Fridovich (1972) [4]. 0.5 ml of tissue homogenate was diluted with 0.5 ml of distilled water, to which 0.25 ml of ice-cold ethanol and 0.15 ml of ice-cold chloroform were added. The mixture was mixed well using a cyclo mixer for 5 minutes and centrifuged at 2500 rpm. To 0.5ml of supernatant, 1.5 ml of carbonate buffer, and 0.5 ml of EDTA solution were added.
- The reaction was initiated by adding 0.4 ml of epinephrine, and the change in optical density/minute was measured at 480 nm against the reagent blank. SOD activity was expressed as units/mg protein. Change in optical density per minute at 50 % inhibition of epinephrine to adrenochrome transition by the enzyme is taken as the enzyme unit.
- The calibration curve was prepared by using 10-125 units of SOD.

3. Determination of Reduced glutathione (GSH)

- Reduced glutathione was determined by the method described by Moron et al. (1979) [5]. Equal volumes of tissue homogenate (supernatant) and 20% TCA were mixed. The precipitated fraction was centrifuged, and to 0.25 ml of supernatant, 2 ml of DTNB reagent was added.

- The final volume was made up to 3ml with phosphate buffer. The color developed was read at 412 nm against the reagent blank. Different concentrations (10-50 gm.) of standard glutathione were taken and processed for the standard graph.
- Reduced glutathione was expressed as µg of GSH / mg protein.

4. Determination of nitric oxide (NO)

- The NO level was estimated as nitrite by the acidic Griess reaction after reducing nitrate to nitrite by vanadium trichloride, according to Miranda et al., 2001.
- The Griess reaction relies on a simple colorimetric reaction between nitrite, sulfonamide, and N-(1- naphthyl) ethylenediamine to produce a pink azo-product with maximum absorbance at 543 nm.
- The concentrations were determined using a standard curve of sodium nitrate, and the results were expressed in µg/mg protein.

5. Determination of tissue protein

- According to the Lowry et al. (1951)[6] method, protein concentration was estimated using BSA (bovine serum albumin) as a standard. Briefly, dilute tissue fraction aliquots (0.1 ml) were taken in a test tube.
- To this, 0.8 ml of 0.1 M sodium hydroxide and 5.0 ml Lowry C reagent was added, and the solution was allowed to stand for 15 min. Then 0.5 ml of Folin phenol reagent was added, and the contents were mixed by vortex mixer. The color developed was measured at 660 nm against reagent blank containing distilled water instead of sample.
- Different concentrations (40-200 µg) of BSA were taken and processed as above for the standard graph.
- The values were expressed as mg of protein/ gm of wet tissue (mg/gm).

2. Determination of brain monoamine

- The brain was isolated immediately and transferred to a homogenization tube containing 5 ml of 0.01 N hydrochloric acid and homogenized. Brain homogenate was transferred to a bottle containing 8 ml of ice-cold absolute alcohol and kept for 1 hour at 0 ºC. The content was centrifuged for 10 min at 16000 rpm, and the supernatant was collected in a Petri dish. The residue was washed with 3-5 ml of 75% alcohol three times, and washes were combined with supernatant. Contents in the Petri dish were evaporated to dryness at 70-90 0C on a water bath under a stream of air. To the dry mass, 1 ml water and 2 ml

chloroform were added and centrifuged at 2000 rpm. The upper phase containing GABA was separated, and 10 ul of it was applied as spot on Whatman paper (No. 41).

- The mobile phase consisted of n-butanol (50 ml), acetic acid (12 ml), and water (60 ml). The chamber was saturated for a half-hour with the mobile phase. The paper chromatogram was developed with ascending technique. The paper was dried in hot air and then spread with 0.5% ninhydrin solution in 95% ethanol. The paper was dried for 1 hr at 90 0C. Blue color spot developed on paper was cut and heated with 2 ml ninhydrin solution on a water bath for 5 min. Water (5 ml) was added to the solution and kept for 1h. The supernatant was decanted, and absorbance was measured at 570 nm.
- A stock solution of standard GABA, 1 mg/ml, was prepared in 0.01N HCl. Serial dilutions were prepared to get concentrations 1ng/10ul to 1000ng/10ul. To obtain a standard concentration curve for GABA same procedure was followed, replacing brain homogenate with standard GABA solutions (Mayn et al., 1962).[7]

3. Determination of brain 5-hydroxytryptamine

- In 2.5 ml of brain homogenate in acidified butanol, 0.1 ml of 0.1 N HCI containing 0.025-10.0/zg of 5HT were added, followed by 0.6 ml of a freshly prepared solution of o-phthalaldehyde (4 mg/100 ml) in 10 N HCI.
- After mixing with a vibrating mixer, the tubes were placed in a boiling water bath for 15 min, then removed and cooled in tap water. Fluorescence was measured in a spectrophotofluorometer. Activation and emission wavelengths were set at 360 nm and 470 nm, respectively, to determine the concentration of 5-HT (Green and Curzon, 1968).[8]

4. Determination of brain dopamine

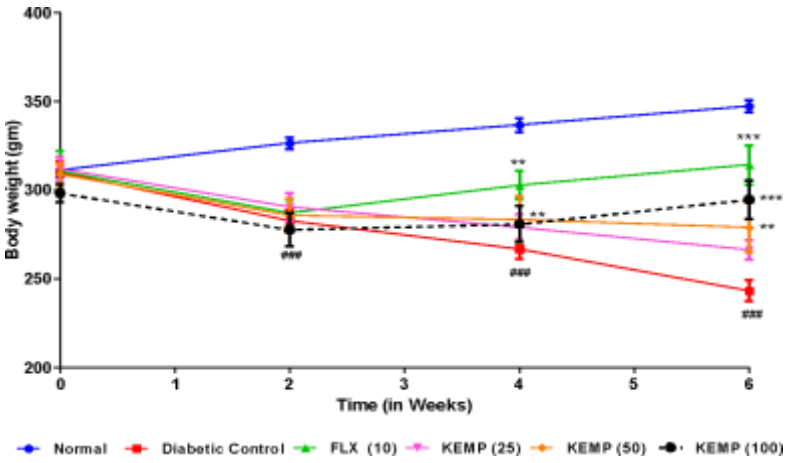
- The oxidation procedure was reported by Fleming et al. (1965)[9] In 1.25 ml of brain homogenate in 0.1 ml of 0.1N HCI were added and 0.25 ml of 1M phosphate buffer added.
- 0.025 ml of 1N iodine in absolute ethanol was added. After mixing, the tubes were allowed to stand exactly two minutes at room temperature, then 0.2 ml of a freshly prepared alkaline sulfite solution (2.5 g of anhydrous Na2SO3 in water and 9 ml of 5N NaOH) was added to each tube.
- The tubes were mixed again, then after exactly 1.5 min, 0.2 ml of 5N acetic acid were added, and the tubes were heated in a boiling water bath for 2 min.

- The fluorescence was measured after cooling in tap water at activation and emission wavelengths of 378 nm and 335 nm, respectively, to determine dopamine concentration (Schlumpf et al., 1974).[10]

III. RESULTS AND DISCUSSION

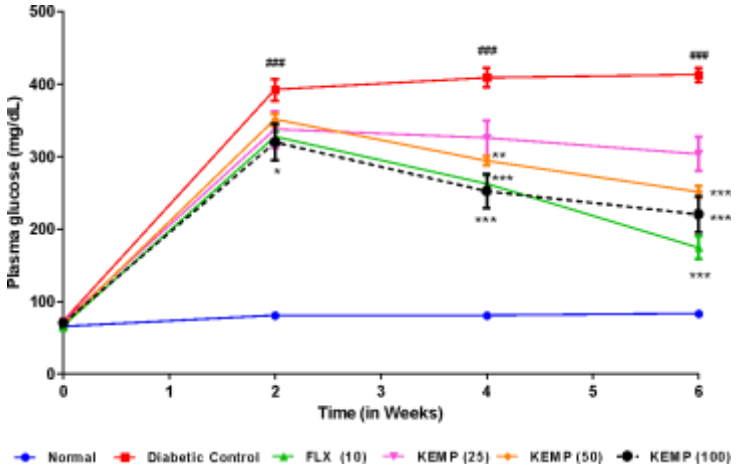
1. Effect of kaempferol on diabetes-induced alteration in body weight.

Time	Normal	Diabetic control	Fluoxetine (10 mg/kg)	KEMP (25mg/kg)	KEMP (50mg/kg)	KEMP (100mg/kg)
0	311.33 ±3.74	309.83 ±6.48	310.50±11.7	312.00 ±6.66	308.83 ±6.38	298.33 ±5.17
2	326.50 ±3.3	282.67±5.97	287.17 ±7.40	290.50 ±7.78	285.67 ±9.64	277.67 ±9.38
4	336.67 ±3.87	266.67±5.43	302.83±8.02	279.00 ±7.36	283.33±13.3	280.83±10.26
6	347.33 ±3.35	243.17±5.98	314.17±10.9	266.33 ±5.43	278.83±13.9	294.67±10.8



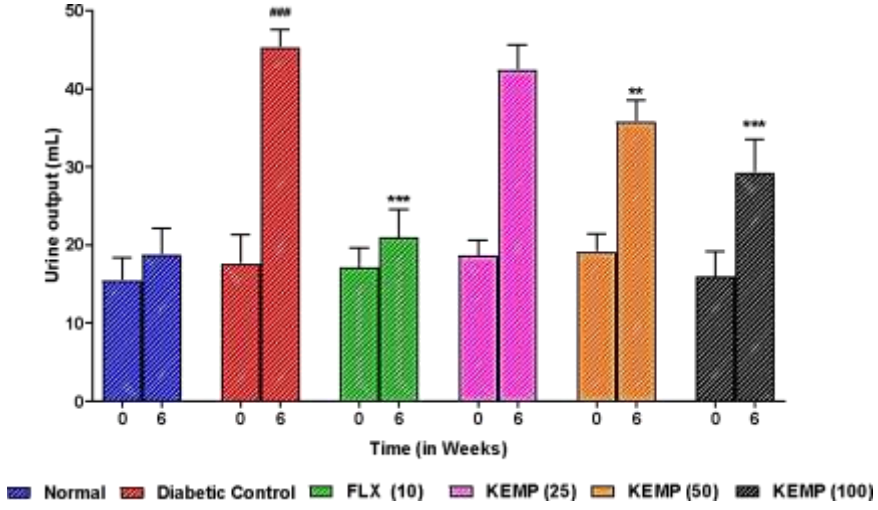
2. Effect of kaempferol on diabetes-induced alteration in plasma glucose levels.

Time	Plasma glucose level(mg/dl)Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10 mg/kg)	KEMP (25 mg/kg)	KEMP (50 mg/kg)	KEMP (100mg/kg)
0	65.33 ±3.93	72.17 ±4.19	66.33 ± 6.44	71.50 ±4.93	68.33 ±5.08	70.33 ± 4.01
2	80.83 ±1.62	392.17 ±15.02	327.33 ± 7.00	337.17 ±25.17	350.83 ±8.66	320.00 ±25.08
4	80.67 ±1.26	409.17 ±13.29	262.67 ±11.41	325.50 ±24.32	294.50 ±6.78	252.33 ±23.60
6	83.00 ±1.06	412.67 ±9.89	174.67 ±15.97	303.67 ±23.71	251.50 ±8.07	220.33 ±24.61



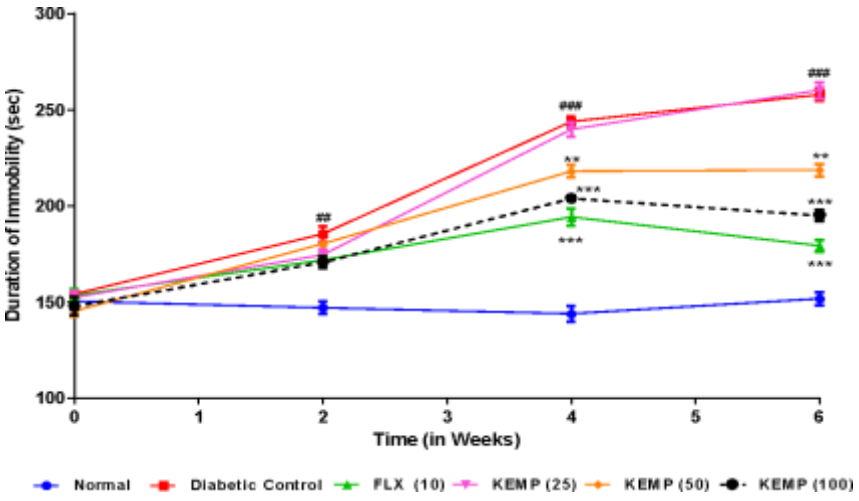
3. Effect of kaempferol on diabetes-induced alteration in Output of urine

Time	Outputofurine(ml)Mean± SEM					
	Normal	Diabetic control	Fluoxetine(10 mg/kg)	KEMP (25mg/kg)	KEMP (50mg/kg)	KEMP (100mg/kg)
0	15.50 ±1.18	17.67 ±1.50	17.17 ± 1.01	18.67 ±0.80	19.17 ±0.91	16.00 ±1.29
6	18.83 ±1.35	45.33 ±0.92	21.00 ±1.46	42.50 ±1.28	35.83 ±1.11	29.33 ±1.73



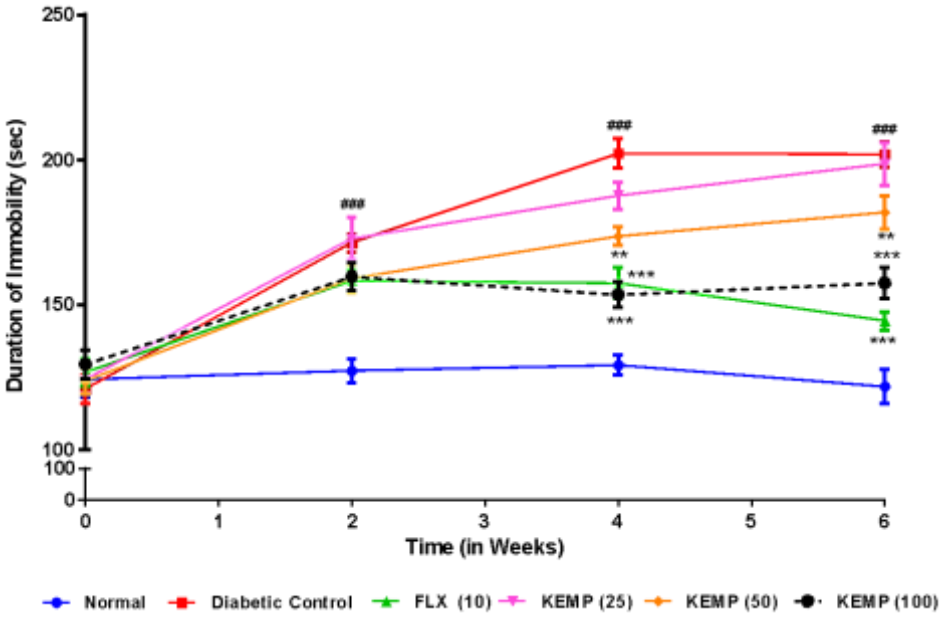
4. Effect of kaempferol on diabetes-induced alteration in duration of immobility during tail suspension test

Time	Duration of immobility(sec)Mean±SEM					
	Normal	Diabetic control	Fluoxetine(10 mg/kg)	KEMP (25mg/kg)	KEMP (50mg/kg)	KEMP (100mg/kg)
0	150.17±3.89	154.17 ±2.70	153.50 ± 3.29	152.50 ±3.55	145.17 ±2.47	148.00±4.70
2	147.17 3.41	185.33 ±4.25	171.83 ± 3.08	174.50 ± 3.21	180.17 ±2.14	170.83 ±3.11
4	143.83±4.08	243.83 ±3.07	194.33 ±4.48	239.83 ± 3.89	218.17 ± 3.32	204.00 ±2.13
6	151.83±3.51	258.00 ±3.21	179.33 ±3.12	260.17 ±4.28	218.67 ±3.42	195.17 ±2.93



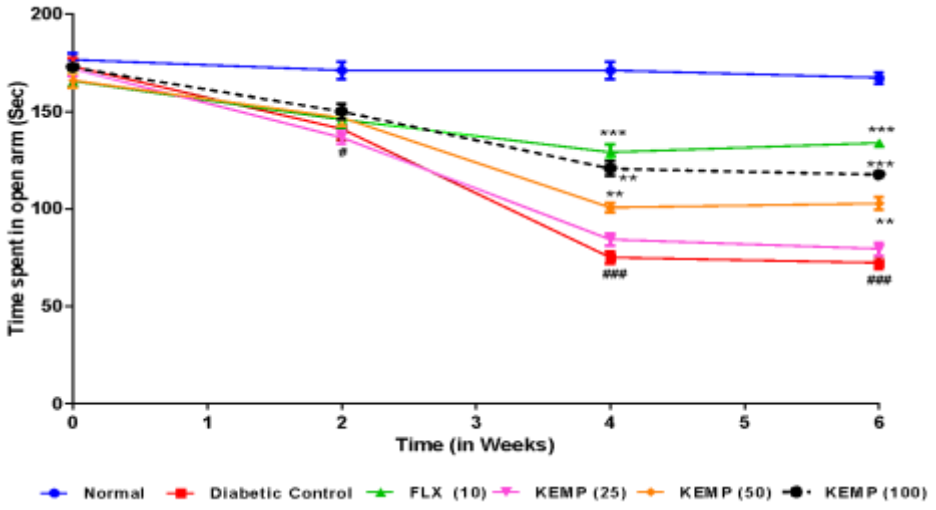
5. **Effect of kaempferol on diabetes-induced alteration in duration of immobility in forced swim test**

Time	Duration of immobility (sec) Mean ± SEM					
	Normal	Diabetic control	Fluoxetine (10 mg/kg)	KEMP (25 mg/kg)	KEMP (50mg/kg)	KEMP (100mg/kg)
0	124.17 ± 6.12	120.83 ± 5.06	126.67 ±4.83	124.17 ± 4.74	123.50 ±4.40	129.50 ± 4.90
2	127.17 ± 4.13	171.33 ± 3.23	158.33 ± 3.68	172.83 ± 7.31	159.00 ± 4.85	159.67 ± 4.86
4	129.17 ± 3.50	202.17 ± 5.06	157.50 ± 5.32	187.67 ± 4.65	173.67 ± 3.14	153.50 ± 4.31
6	121.83 ± 5.86	201.83 ± 4.30	144.33 ± 3.13	198.50 ± 7.49	181.83 ± 5.72	157.50 ± 5.40



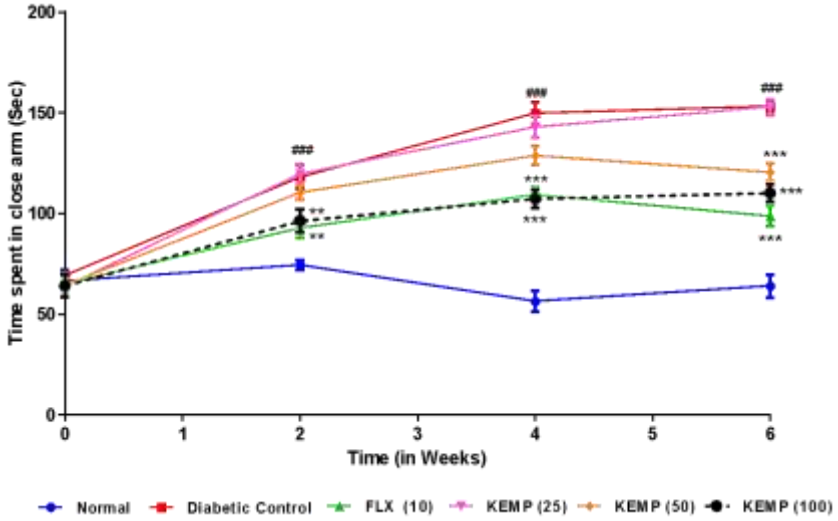
6. Effect of kaempferol on diabetes-induced alteration in time spent in open arm in the elevated plus-maze test

Time	Time spent in open arm (Sec) Mean ± SEM					
	Normal	Diabetic control	Fluoxetine (10 mg/kg)	KEMP (25mg/kg)	KEMP (50mg/kg)	KEMP (100mg/kg)
0	176.67 ± 3.11	173.17 ± 4.36	165.50 ± 2.83	171.67 ± 3.35	166.00 ± 3.75	172.67 ± 2.11
2	171.17 ± 4.46	140.83 ± 5.18$^{\#}$	145.83 ± 4.71	136.67 ± 3.32	146.83 ± 4.28	150.00 ± 4.02
4	171.17 ± 4.47	74.83 ± 3.09	129.17 ± 4.01	84.17 ± 3.05	100.67 ± 2.32	120.67 ± 3.81
6	167.17 ± 2.80	72.33 ± 3.23	133.83 ± 1.45	79.33 ± 3.47	102.83 ± 3.36	117.67 ± 2.28



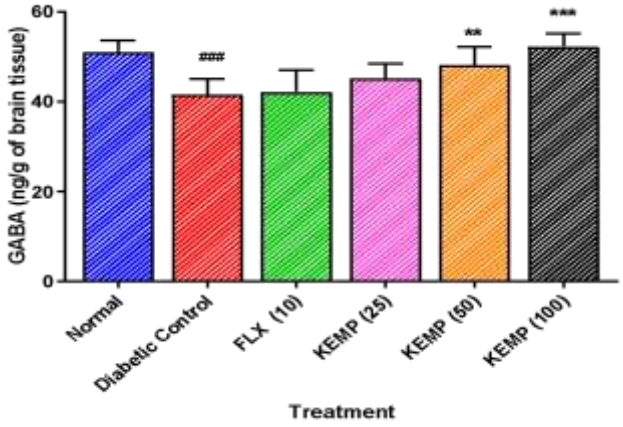
7. Effect of kaempferol on diabetes-induced alteration in time spent in closed arm in the elevated plus-maze test

Time (in week)	Time spent in the closed arm (Sec) Mean ± SEM					
	Normal	Diabetic control	Fluoxetine (10 mg/kg)	KEMP (25 mg/kg)	KEMP (50 mg/kg)	KEMP (100 mg/kg)
0	66.50 ± 5.46	69.17 ± 2.06	65.17 ± 5.06	63.83 ± 4.09	64.50 ± 2.59	64.17 ± 5.67
2	74.33 ± 2.54	118.17 ± 5.78	92.83 ± 4.76	119.67 ± 5.04	110.33 ± 3.42	96.33 ± 5.78
4	56.33 ± 5.21	149.83 ± 5.63	109.50 ± 3.78	143.00 ± 5.29	128.83 ± 4.58	107.33 ± 4.48
6	64.00 ± 5.74	153.17 ± 2.80	98.67 ± 5.05	152.83 ± 3.96	120.50 ± 4.38	110.17 ± 4.38



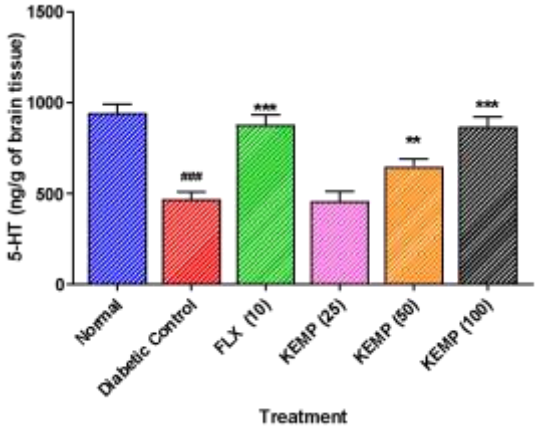
8. Effect of kaempferol on diabetes-induced alteration in brain GABA levels

Brain GABA (ng/g of brain tissue) Mean ± SEM					
Normal	**Diabetic control**	**Fluoxetine (10 mg/kg)**	**KEMP (25 mg/kg)**	**KEMP (50 mg/kg)**	**KEMP (100 mg/kg)**
51.12 ± 1.03	41.60 ± 1.46	42.23 ± 1.99	45.24 ± 1.36	48.23 ± 1.65	52.31 ± 1.21



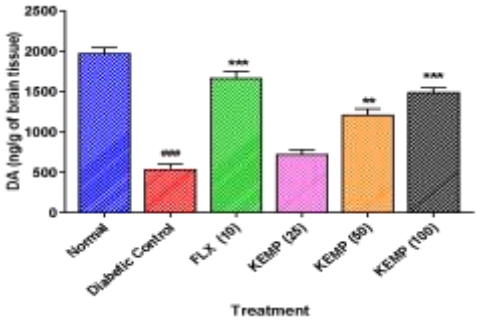
9. Effect of kaempferol on diabetes-induced alteration in brain 5-HT levels

Brain 5-HT (ng/g of brain tissue) Mean ± SEM					
Normal	**Diabetic control**	**Fluoxetine (10mg/kg)**	**KEMP (25mg/kg)**	**KEMP (50mg/kg)**	**KEMP (100mg/kg)**
945.70 ± 18.71	470.60 ± 15.65	882.20 ± 21.53	460.40 ± 20.82	650.9± 16.42	870.9± 21.66



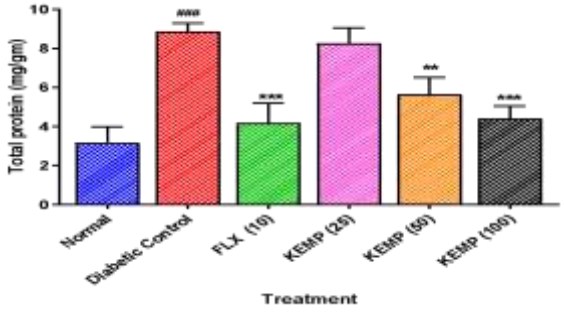
10. Effect of kaempferol on diabetes-induced alteration in brain dopamine levels

Dopamine levels in brain (ng/g of brain tissue) Mean ± SEM					
Normal	**Diabetic control**	**Fluoxetine (10 mg/kg)**	**KEMP (25mg/kg)**	**KEMP (50mg/kg)**	**KEMP (100 mg/kg)**
1983.00 ± 26.07	544.50 ± 23.07	1675.00 ± 32.26	730.00 ± 20.36	1216.00 ± 28.30	1498.00 ± 20.22



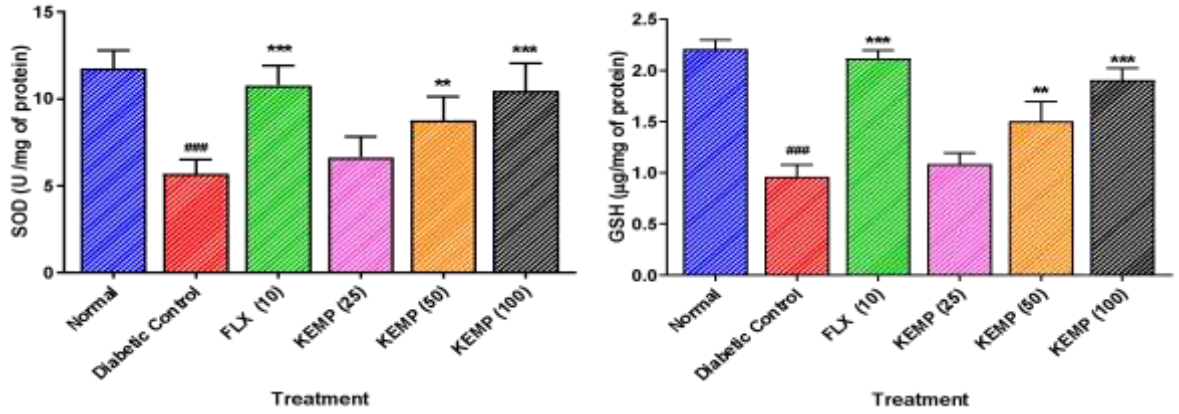
11. Effect of kaempferol on diabetes-induced alteration in total protein levels in brain level

Total protein levels in brain (mg/gm) Mean ± SEM					
Normal	**Diabetic control**	**Fluoxetine (10 mg/kg)**	**KEMP (25 mg/kg)**	**KEMP (50 mg/kg)**	**KEMP (100 mg/kg)**
3.18 ± 0.33	8.88 ± 0.17	4.20 ± 0.41	8.28 ± 0.32	5.65 ± 0.35	4.41 ± 0.26



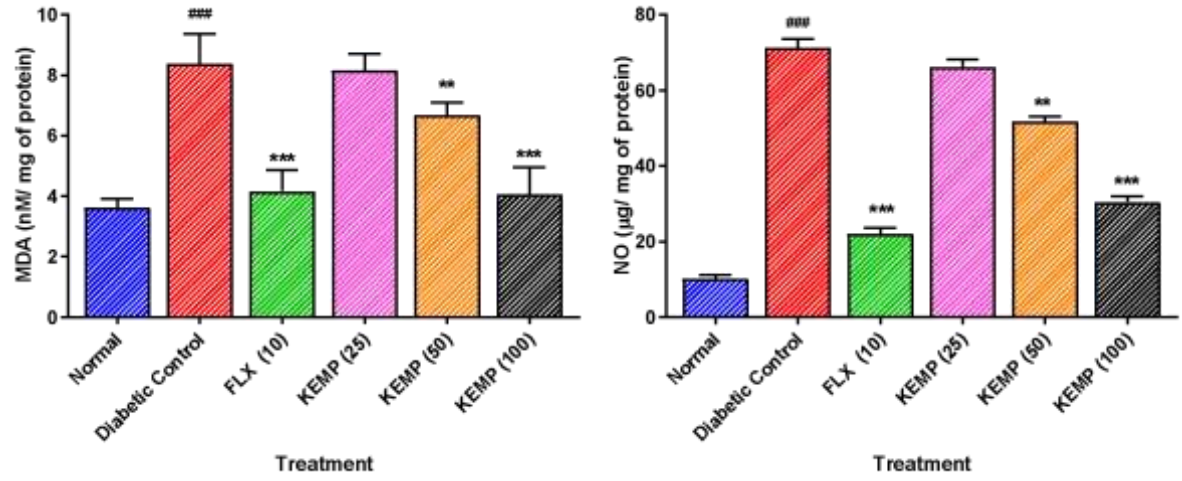
12. Effect of kaempferol on diabetes-induced alteration in brain SOD and GSH level:

Parameter	Brain SOD (U /mg of protein) and GSH µg/mg of protein) levels - Mean ± SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	KEMP (25mg/kg)	KEMP (50mg/kg)	KEMP (100mg/kg)
SOD (U /mg of protein)	11.77 ± 0.42	5.69 ± 0.33	10.78 ± 0.46	6.64 ± 0.49	8.76 ± 0.57	10.46± 0.65
GSH (µg/mg of protein)	2.21 ± 0.03	0.96 ± 0.05	2.12 ± 0.03	1.09 ± 0.04	1.51 ± 0.08	1.91 0.05



13. Effect of kaempferol on diabetes-induced alteration in brain MDA and nitric oxide level

Parameter	Brain MDA (nM/mg of protein), nitric oxide (µg/ mg of protein) Mean ± SEM					
	Normal	Diabetic control	Fluoxetine (10 mg/kg)	KEMP (25 mg/kg)	KEMP (50 mg/kg)	KEMP (100 mg/kg)
MDA (nM/mg of protein)	3.62 ± 0.12	8.38 ± 0.40	4.16 ± 0.29	8.16 ± 0.23	6.68 ± 0.17	4.06 ± 0.36
Nitric oxide (µg/ mg of protein)	10.18 ± 0.42	71.37 ± 0.94	22.02 ± 0.65	66.25 ± 0.79	51.81 ± 0.52	30.48 ± 0.63



DISCUSSION

Diabetes is a metabolic disorder worldwide; epidemiological study suggest that prevalence both category male & female. According to World Health of Organization (WHO) there are two type of diabetes mellitus i.e. short term & long term. Short term is called as Type 1 diabetes & long term is called as Type 2 diabetes. Type 2 is again divided into 2 forms micro vascular complication & macro vascular complication.

Kaempferol exhibits its antidepressant and anxiolytic effects through a multifaceted mechanism involving antioxidant and anti-inflammatory activities, modulation of neurotransmitter levels, improvement in glucose metabolism, and protection against neuronal damage. These combined effects make kaempferol a promising therapeutic agent for managing depression and anxiety in diabetic conditions.

In present study we carried out in vivo parameters Body weight, food intake, water intake, and urinary output, elevated plus maze test, plasma glucose levels, Number of open arm entries, Number of closed arm entries, Time spent in open arm, Time spent in closed arm, Time spent in a central square, forced swimming test, Tail suspension test, Ex-vivo parameters, Determination of brain oxidative stress, Oxidative stress (SOD, GSH, MDA, Nitric oxide and Total Protein) in brain, Brain GABA levels.

In present study effect of kaempferol on diabetes-induced alteration showed body weight 311.33 ± 3.74 at 0 week & 2nd week it showed 326.50 ± 3.3 & on last week of study we found 347.33 ± 3.35. Hence vehicle treated animal prevent loss of fatty material. In 2nd gr of diabetic control rat showed body weight 309.83 ± 6.48 on 0 week & on last week alloxan causes degradation of fatty materials. Fluoxetine std drug 10mg/kg showed significant increase in body weight on last date of study period test gr kaempferol found 100mg/kg from significant prevent loss of fatty materials because of result of last day of study period.

Second parameter effect of kaempferol on diabetes-induced alteration Showed plasma glucose levels 65.33 ± 3.93 at 0 week & 2nd week it showed decrease 80.83 ± 1.62 on last week of study we found 83.00 ± 1.06. In 2nd gr of diabetic control rat showed glucose level 72.17 ± 4.19 on 0 week & on last week alloxan causes increase in plasma glucose levels. Fluoxetine std drug 10mg/kg showed significant increase in plasma glucose levels on last date of study period test gr kaempferol found 100mg/kg failed to produce any effective decrease in serum glucose level.

Parameter effect of kaempferol on diabetes-induced alteration showed Intake of food 23.07 ± 0.66 on 0 week & on last week it was 25.23 ± 1.65. There was no prominent difference in the intake of food of diabetic control rats than the non-diabetic rats on week 0. The alloxan-treated control group showed an evidently elevated intake of food than normal rats on the 6th week. There was a prominent and dose-dependent decrease) in the amount of intake of food on treatment with Kaempferol at a dose of 50 and 100 mg/kg as compared with diabetic control groups. When compared with diabetic control rats, fluoxetine at a dose of 10 mg/kg treated rats also showed an effective decrease in the intake of food on the 6th week.

Parameter effect of kaempferol on diabetes-induced alteration showed Intake of water 53.17 ± 4.45 on 0 week & on last week it was 54.17 ± 3.33. There was no meaningful difference in the intake of water of diabetic control rats before induction of diabetes on day 0 than normal rats. Diabetic control rats showed evidently elevated intake of water than the normal rats. Kaempferol at a dose of 50 and 100 mg/kg showed prominent, and dose-dependent lessened in intake of water than diabetic control rats. Additionally, treatment with fluoxetine at a dose of 10 mg/kg showed an effective decrease in intake of water than the diabetic control rats in the 6th week.

Parameter effect of kaempferol on diabetes-induced alteration showed Output of urine 15.50 ± 1.18 on 0 week & on last week it was18.83 ± 1.35. There was no prominent difference in the output of urine of the normal rat than diabetic control rats on day 0 before induction of diabetes. On week 6, diabetic control rats showed evidently amplified output of urine than the normal rats. Whereas treatment with Kaempferol at a dose of 50 and 100 mg/kg showed evidently and dose-dependent lessened in output of urine than the diabetic control rats. Fluoxetine at a dose of 10 mg/kg treated group showed a meaningful decrease in output of urine as compared with diabetic control rats in the 6th week.

Parameter effect of kaempferol on diabetes-induced alteration showed duration of immobility in tail suspension test, 150.17 ± 3.89 there was effective elevated duration of immobility of diabetic control rats after 2nd week of alloxan administration than normal rats. Kaempferol at a dose of 50 and 100 mg/kg evidently attenuated this amplified immobility duration than diabetic control rats from 4th week onwards. Treatment with fluoxetine at a dose of 10 mg/kg also evidently ameliorated the elevated immobility duration than diabetic control rats from 4th week onwards.

Parameter effect of kaempferol on diabetes-induced alteration showed duration of immobility in forced swim test, the duration of immobility was evidently elevated in the diabetic control rats on 2nd weeks of intraperitoneal administration of alloxan than normal non-diabetic rats. Treatment with Kaempferol at a dose of 50 and 100 mg/kg evidently and dose-dependently ameliorated this amplified in immobility duration than diabetic control rats on respective days. Fluoxetine at a dose of 10 mg/kg also showed the evidently attenuation in amplified duration of immobility than diabetic control rats.

Parameter effect of kaempferol on diabetes-induced alteration in time spent in open arm in the elevated plus-maze test, Time spent by the diabetic control rats in the open arm of the elevated plus-maze was evidently lowered after 2nd week of intraperitoneal administration of alloxan than normal non-diabetic rats. Time duration spent in the open arm by the diabetic control rats is still evidently reduced up to the 6th week than the 2nd week. After 6 weeks of oral administration of Kaempferol (50 and 100 mg/kg), the time spent in the open arm was evidently and dose-dependently elevated than diabetic control rats on respective weeks. This open arm time duration was evidently elevated by fluoxetine at a dose of 10 mg/kg treatment on 3rd week onwards than diabetic control rats.

Parameter effect of kaempferol on diabetes-induced alteration in time spent in closed arm in the elevated plus-maze test, by the diabetic control rats was evidently higher than the normal non-diabetic rats from 2nd week onwards. This elevated in the time duration of close arm was evidently attenuated by Kaempferol at a dose of 50 and 100 mg/kg treatment on 6th and 2nd week onwards than diabetic control rats. Fluoxetine at a dose of 10 mg/kg also showed the prominent reduced in close arm time spent than diabetic control rats from 2nd week onwards.

Parameter effect of kaempferol on diabetes-induced alteration in brain GABA levels, there was an effective decrease in the level of brain GABA after six weeks of intraperitoneal alloxan administration in diabetic control rats than normal rats. This decrease in brain GABA level did not evidently restore by either Kaempferol at a dose of 25 mg/kg or fluoxetine at a dose of 10 mg/kg compared with diabetic control rats. However, Kaempferol at a dose of 50 and 100 mg/kg treatment showed a significant and dose dependant elevation in the levels of brain GABA than diabetic control rats.

Parameter effect of kaempferol on diabetes-induced alteration in brain 5-HT levels, compared with normal non-diabetic control rats, diabetic control rats showed evidently lessened in the

brain 5-HT level on the 6th week of alloxan administration. This reduced level of brain 5-HT was evidently amplified by the treatment of Kaempferol at a dose of 50 and 100 mg/kg than diabetic control rats. Whereas fluoxetine at a dose of 10 mg/kg treatment also evidently elevates, this lessened brain 5-HT levels than diabetic control rats.

Parameter effect of kaempferol on diabetes-induced alteration in brain SOD and GSH level, there was a meaningful decrease in levels of SOD and GSH in brain of the diabetic control rats than the normal rats. Treatment with Kaempferol at a dose of 50 and 100 mg/kg show prominent, and dose dependant amplified in levels of SOD and GSH in brain than the diabetic control rats. Treatment with fluoxetine at a dose of 10 mg/kg also showed a meaningful raise in brain SOD as well as GSH levels than the diabetic control rats.

Parameter effect of kaempferol on diabetes-induced alteration in brain MDA and nitric oxide level, compared with normal rats, diabetic control rats showed evidently elevated levels of MDA and NO in brain. There was an effective and dose-dependent decrease in levels of MDA and NO in brain by treatment of Kaempferol at a dose of 50 and 100 mg/kg than diabetic control rats. Fluoxetine at a dose of 10 mg/kg treated rats also show prominent decreases in levels of MDA and NO in brain than diabetic control rats.

CONCLUSION

The findings of this study suggest that kaempferol has significant antidepressant-like effects in an alloxan-induced diabetic rat model. These effects are supported by the following observations:

1. Prevention of Weight Loss

Kaempferol prevented the weight loss typically observed in diabetic rats, indicating its ability to protect against metabolic disturbances associated with diabetes.

2. Behavioral Improvements

Kaempferol administration resulted in reduced immobility in both the forced swim test and tail suspension test, reflecting its potential to alleviate depressive-like symptoms. The increase in time spent in the open arm of the elevated plus-maze test suggests an anxiolytic effect of kaempferol.

3. Biochemical Restorations

Kaempferol significantly improved oxidative stress markers (SOD, GSH) and reduced harmful oxidative markers (MDA, nitric oxide). It also restored neurotransmitter levels (GABA, 5-HT), which are crucial for maintaining normal brain function and mood regulation.

4. Comparison with Fluoxetine

The effects of kaempferol were comparable to those of fluoxetine, a standard antidepressant, further validating its potential therapeutic benefits.

5. Potential Clinical Relevance

Given the widespread prevalence of diabetes and the associated risk of depression, kaempferol could serve as a dual therapeutic agent to manage both conditions. Its ability to modulate oxidative stress and neurotransmitter levels highlights its potential as a novel treatment strategy.

6. Future Research

Further studies, including clinical trials, are necessary to confirm the efficacy and safety of kaempferol in diabetic patients with depression.

In summary, this study demonstrates that kaempferol possesses significant antidepressant and neuroprotective effects in a diabetic rat model, making it beneficiary for future research and potential clinical application. The study performed on Kaempferol suggest that Kaempferol possess good properties of being a Drug and has a low toxicity profile. The study carried out further suggest that Kaempferol can acts on diabetes through the mechanism of depression or by oxidative tissue damage. The above observation of the study confirms that the animals have been induced with diabetes.

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