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Original Research Article

“We are antidepressant also”: aspirin and diclofenac sodium

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ABSTRACT

Background: Many studies have indicated that inflammation and depression are associated with each other. Present study was taken up to prove antidepressant effect of aspirin and diclofenac sodium.

Methods: The present study was divided into 6 phases with 5 groups of animals N=10. In study 1, C57Bl mice were used and in remaining 4 studies swiss albino mice. Amitriptyline was standard drug used. For each study first group of animals was treated with a saline solution 1ml P.O., and second group of animals injected with 0.1 ml of 2% formalin. In group 3, 4, 5 animals depression was produced by stressors and treated with aspirin 14mg/kg P.O., diclofenac sod. 10 mg/kg P.O. and amitriptyline 10 mg/kg P.O., respectively. Antidepressant activity of aspirin and diclofenac sodium was determined by using forced swim test, tail suspension test, elevated plus maze test and light dark box test. Inflammatory mediators (IL-6, TNF- α) and central neurotransmitters (5-HT, NE, Ach) were estimated.

Results: In light dark box test, latency of first crossing, time spent in dark area were decreased and no. of crossing increased significantly in the aspirin, diclofenac sod. treated animals. In forced swim test, the immobility time was decreased. Swiss albino mice treated with aspirin, diclofenac sod. showed decreased concentration of IL-6 and TNF- α and increased concentration of serotonin, nor epinephrine and acetylcholine. In elevated plus maze test, no. of open arm, closed arm entries, time spent in open arm increased and time spent in closed arm decreased. In tail suspension test immobility time was decreased.

Conclusions: Aspirin and diclofenac sodium has antidepressant activity.

Keywords: Depression, Aspirin, Diclofenac sodium, Amitriptyline, Cytokines, Central neurotransmitters

INTRODUCTION

Depression is a psychological disorder characterized by disturbed mood and loss of interest, disturbed sleep, reduced appetite and the low energy level. There are 3 primary types of depression 1) bipolar disorder - It includes the mania or hypomania means abnormal elevation in mood. 2) major depressive disorder - In this subject having the complications about work, sleep, appetite and happiness. 3) persistent depressive disorder – Having 2 years or more than 2 years episodes of major depression.^{1,2}

Inflammation is the complex reaction of living vascularized cells to infective agents. This response

avoids the tissue to be destroyed.³ It is a defence mechanism of living organisms. Inflammation is a part of immune system; which self-protect the body and removes harmful stimuli.⁴

One of the important causes of depression is decreased level of central neurotransmitters and increased level of inflammatory mediators. In inflammation the leukocytes such as lymphocytes, neutrophils and macrophages release the vasoactive amines, peptides, cytokines (interleukin-1, interleukin-6, interleukin-12, tumor necrosis factor) and eicosanoids. These substances mediate the inflammation process therefore these are called as inflammatory mediators.^{5,6} A chemical substance released at the nerve fiber end, which transfer

nerve impulses from one nerve fiber to another is called as neurotransmitters.⁷ Aspirin and diclofenac sodium as Non-Steroidal Anti-Inflammatory drugs decreases the level of inflammatory mediators. Interleukin, an inflammatory mediator decreases the concentration of nor-epinephrine in the hypothalamus.^{8,9} This decreased concentration of nor-epinephrine can cause depression. Interleukin-1 β also decreases the secretion of acetylcholine.⁸ The increased concentration of cytokines decreases the level of serotonin.¹⁰ Many studies have indicated that inflammation and depression are associated with each other. It is hypothesizing that the anti-inflammatory drugs such as aspirin and diclofenac sodium have an antidepressant activity and hence this study is taken up.

METHODS

Animals

50 male C57Bl-6 mice weighing 25-30 gm, procured from Invivo Biosciences, Kodigehalli village, Bengaluru 560091 (Reg. no. 1165/Po/ReBiBt-5/NRC-L/08/CPCSEA). And 50 male Swiss albino mice, weighing 30-35 gm, procured from Biogen, Laboratory Animal facility, Attibele, Bengaluru-562107 (Reg. No. 971/Po/RcBiBt/s/2006/CPCSEA), were acclimatized for 7 days at normal laboratory condition, maintained at room temperature, 12 hr. light/dark cycle with water ad libitum and food provided twice a day. Before conducting the experiments, permission was obtained from "institutional animal ethics committee", Visveswara pura Institute of Pharmaceutical Sciences, Bengaluru. (IAEC No:152/PO/ReBi/S/99/CPCSEA).

Drugs and chemicals

The pure form of drugs Aspirin and Amitriptyline was purchased from yarrow chem products company, Mumbai, India. Diclofenac sodium was obtained from Cipla Ltd. Maharashtra, India. Mouse interleukin-6 ELISA kit and Mouse TNF- α ELISA kit was obtained from Bioassay Technology Laboratory, Shanghai, China. All other chemicals were obtained from institutional chemical vendor.

Grouping and treatment

The research was divided into 6 studies. Every study included 5 groups of animals (n=10). In study 1, C57Bl mice were used and in remaining 4 studies swiss albino mice were used. First group of animals of every study was treated with a saline solution 1 ml P.O. Inflammation was produced in second group of animals of every study by injecting 0.1 ml of 2% formalin. In group 3, 4, 5 animals of every study depression were produced by stressors. Third, fourth and fifth group of animals of every study were treated with aspirin 14 mg/kg P.O.,

diclofenac sod. 10 mg/kg P.O. and amitriptyline 10 mg/kg P.O., as a standard respectively.¹¹⁻¹³

Study 1 was effect of aspirin, diclofenac sodium and Amitriptyline as an antidepressant drug on latency of first crossing, no. of crossing and time spent in dark area using light dark box test. Study 2 was the effect of aspirin, diclofenac sodium and amitriptyline as an antidepressant drug on immobility time using Forced swim test. Study 3: estimation of inflammatory mediators from blood sample of swiss albino mice treated with aspirin, diclofenac sodium and amitriptyline. Study-4 was estimation of neurotransmitters from brain homogenate of swiss albino mice treated with Aspirin, Diclofenac sodium and amitriptyline. Study-5 was effect aspirin, diclofenac sodium and amitriptyline as an antidepressant drug on open arm entries, closed arm entries, open arm duration, closed arm duration in swiss albino mice using elevated plus maze test. Study-6 was effect of aspirin, diclofenac sodium and amitriptyline as an antidepressant drug on immobility time in swiss albino mice using tail suspension test.

Disease control animal models

Formalin induced mice paw edema model

Plethysmometer was used to measure paw volume in mice. Mice in all the studies were injected with 0.1 ml of 2% formalin in the sub planter area of right hind paw. Paw volume of mice of both legs was measured at 0, 30, 60 min. by using plethysmometer.¹⁴

Chronic mild stress model

Depression was produced by exposing the mice to the series of different stressors. The animal was placed in 12 \times 3 cm plastic tube (falcon tube). The tube was closed with cap, so the animal is unable to move outside. The 1 cm hole was created at the end of the tube to allow regular breathing. Animal was kept for 3 hrs in this tube. After 3 hrs animal was removed from tube and placed in a cage alone for 24 hrs without food and water. The neurotransmitter level will decrease due to stressors and animal gets depressed.¹⁵

Light dark box test

Light dark box test was used for screening locomotor activity in animals. Hebb William maze apparatus was used for light dark box test. It was a rectangular box which was divided into 2 compartments. 1/3rd was for the dark compartment and 2/3rd was served as light compartment. Treatment was given as mentioned above. After 1 hr of drug administration, each mouse was placed in light compartment, latency to the first crossing to the dark compartment, number of crossing between the light and dark area, total time spent in the dark area of apparatus was noted during the test session of 5 min.¹⁶

Forced swim test

Forced swim test was developed as a rodent screening test for the antidepressant drugs. A 1000 ml glass transparent beaker was filled with tap water at 23°C. The depth of water was adjusted according to the sizes of mice so that mice couldn't land at the bottom of beaker. Two parts of this test were carried out, an initial training period of 15 min. before drug treatment, and second part, after 24 hrs of drug treatment. Each mouse was placed in the water filled beaker for 15 min. before drug treatment. After drug treatment same procedure was carried out after 24 hrs. All the animals were subjected for swimming on day 1, day 7, day 14 and day 21. The immobility time for all animals were noted.¹⁷

Estimation of IL-6 and TNF- α

After 21 days of drug treatment blood was collected from mice in heparinized tube by using retro-orbital method. Blood plasma was separated. Standard solution was prepared by mixing 2% (w/v) standard IL-6 and 2% (w/v) standard TNF- α in wash buffer solution. Sample solution was prepared by diluting blood plasma: phosphate buffered saline 1:10 (10 μ l of sample into 90 μ l of dilution buffer). The whole assay was performed at room temperature. The 7 strips with 56 wells were separated and inserted into the frame. After that 50 μ l standard solution was added into the standard wells and 40 μ l of sample solution was added into the sample wells. Then 10 μ l of anti-IL-6 antibody and TNF- α was added respectively into the sample wells and 50 μ l of streptavidin-HRP solution was added into the all wells. All wells were mixed properly, plates were covered with sealer and incubated for 60 min. at 37°C. After incubation plates washed 5 times using washing buffer and soaked with approximately 0.35ml washing buffer for 30-40 sec. for each wash. Plates were blotted onto the paper for absorbing and 50 μ l of avidin substrate solution (solution A) and biotin substrate solution (solution B) were added in each well. All plates were covered with sealer and incubated for 10 min. at 37°C in the dark room. 50 μ l of stop solution (2 M sulfuric acid) was added into each well. Blue color changes into yellow. Optical density was determined at 450 nm using microplate reader.^{18,19}

Estimation of serotonin

At the end of 21-days drug treatment, mice were sacrificed by cervical dislocation method, dissected and brain was removed. The hypothalamus, Thalamus and cerebral cortex area were dissected. The Serotonin was extracted in n-butanol from the tissue homogenate and transferred into the buffer medium. Tissue was homogenized in 6 ml of 0.4 N Perchloric acid, centrifuged and Supernatant was collected and repeated the centrifugation with 2 ml of 0.4 N Perchloric acid and the supernatant was collected. PH of supernatant was adjusted by adding 10 N NaoH and 0.5 ml of 0.5 M Borate buffer. The mixture was saturated with NaCl and

10 ml of n-butanol was added. Tubes were shaken for 5 min. by vortex mixture and aqueous layer was removed by aspirator. The organic phase was shaken for 2 min. with 2 ml of 0.1 M borate buffer. 8 ml of butanol layer was placed in a separate tube containing 4 ml of 0.05 M phosphate buffer and 10 ml n-heptane. After 2 min. shaking organic phase was removed. 3.6 ml of buffer layer was taken in a tube containing 0.3 ml of 0.1 M Ninhydrin solution. The tubes were incubated at 37°C for 30 min, cooled to Room temperature and the fluorescence was measured in a spectrofluorometric at 385/490 nm excitation and emission.²⁰

Estimation of nor-epinephrine

At the end of 21-days drug treatment, mice were sacrificed by cervical dislocation and brain was homogenized in 5 volumes of ice-cold 0.4 N Perchloric acid and centrifuged at 17000 g for 10 min. The supernatant was collected and used for measuring the total tritium present. The pH was adjusted up to 8.6. The fluorescence was measured in a spectro fluometry at 395/505 nm excitation and emission.²¹

Estimation of Acetylcholine

At the end of 21-days drug treatment, mice were sacrificed by cervical dislocation method, dissected and brain was removed. The corpora striata was dissected. Cells were homogenized in 19 volumes of 0.05 M Phosphate buffer PH 7.2 using a homogenizer. The aliquots were incubated for 10 min. at 37°C. The fluorescence was measured in a spectrofluometry at 395/490 nm excitation and emission.²¹

Elevated plus maze test

The principle behind the elevated plus maze test is that the animals administered with the antidepressant drugs spent more time in open arm on apparatus. The Elevated plus maze apparatus or open arm close arm apparatus was used for this test. Mice were placed in apparatus for 5 min. before drug treatment for exploring the apparatus. Mice were removed from elevated plus maze apparatus and place in home cage. After 15 min. of drug treatment again mice were placed in apparatus and observed for 5 min. Open arm entries, closed arm entries, open arm duration, closed arm duration were measured.²²

Tail suspension test

Tail suspension test is a variant of the behavioural test in which immobility period is measured. Antidepressant drugs reduce the immobility period. The rectangular Rota rod apparatus with 4 compartments was used for this test. The mice were suspended in each separate compartment 50 cm above the floor by the adhesive tape placed approximately 1cm from the tip of the tail. Immobility time was recorded for 5 min. before drug treatment. After drug treatment mice was observed for 5 min. on day 1,

day 7, day 14 and day 21. Immobility time was noted for the animals.²³

Statistical analysis

Data was expressed as Mean±Standard Error of Mean (SEM). Statistical analysis was done by Analysis of Variance (ANOVA) followed by post hoc Dunnett’s test. Graph pad prism software was used for statistical analysis. p-value less than 0.05 were considered as statistically significant.

RESULTS

Light dark box test

In the light dark box test the latency of first crossing was decreased no. of crossing increased and time spent in dark area decreased significantly in aspirin, diclofenac sod. treated groups on day 21 when compared with the disease control group and are comparable with that of standard drug amitriptyline treated animals (Figure 1).

Forced swim test

In the forced swim test immobility time was decreased significantly in the aspirin, diclofenac sod. treated animals as shown in (Table 1) on day 21 when compared with the disease control group and are comparable with that of standard drug amitriptyline treated animals.

Table 1: Effect of Aspirin, Diclofenac Sodium and Amitriptyline on immobility time on day-21 in swiss albino mice using forced swim test.

S. no.	Group	Treatment	Immobility time (sec.)
1.	Control	Saline solution 1 ml	125.1±12.12
2.	Disease control	Formalin injection 0.1 of 2%	178.9±4.108 ⁺
3.	Aspirin	14 mg/kg dissolved in warm water	104.2±4.263 ^{***}
4.	Diclofenac sodium	10 mg/kg dissolved in dis. Water	107.3±6.655 ^{***}
5.	Amitriptyline	10 mg/kg dissolved in dis. Water	105.5±6.424 ^{***}

N=10, Values expressed as Mean ±SEM, one-way ANOVA, followed by post hoc Dunnett’s test, ^{***}p<0.001 V/S disease control and ⁺p<0.01 V/S control group.

Estimation of IL-6 and TNF-α

The conc. of IL-6 and TNF-α were decreased significantly in the aspirin, diclofenac sod. treated groups after 21 days drug treatment when compared with the

disease control group and are comparable with that of standard drug amitriptyline treated animals (Figure 2).

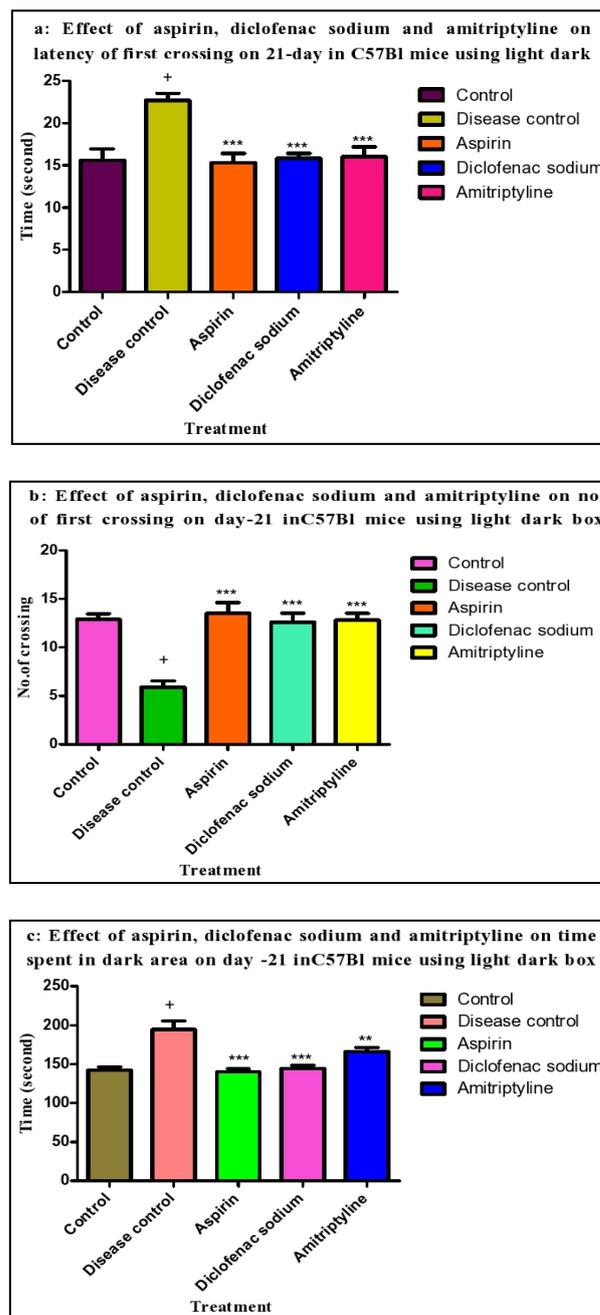


Figure 1: Effect of aspirin, diclofenac sodium and amitriptyline on a) latency of first crossing b) no. of crossing c) time spent in dark area on day-21 in C57Bl mice using light dark box test. N=10, Values expressed as Mean±SEM, one-way ANOVA, followed by post hoc Dunnett’s test, ^{*}p<0.001 V/S disease control and ⁺p<0.01 V/S control group.**

Estimation of central neurotransmitters (Serotonin, Nor-epinephrine and Acetylcholine)

The concentration of serotonin nor-epinephrine and acetylcholine increased significantly in the aspirin,

diclofenac sod. treated groups after 21 days drug treatment when compared with the disease control group and were comparable with the results in the standard drug amitriptyline treated animals (Figure 3).

Elevated plus maze test

In the elevated plus maze test no. of open arm entries was increased, no. of closed arm entries was increased, time spent in open arm was increased, and time spent in closed arm was decreased, significantly in aspirin, diclofenac sod. treated group animals on day 21 when compared with the disease control group. Aspirin, diclofenac sodium treated animals showed statistically significant changes in no. of open arm entries, no. of closed arm entries, time spent in open arm, time spent in closed arm and these changes were comparable with standard drug amitriptyline treated animals in elevated plus maze test (Figure 4).

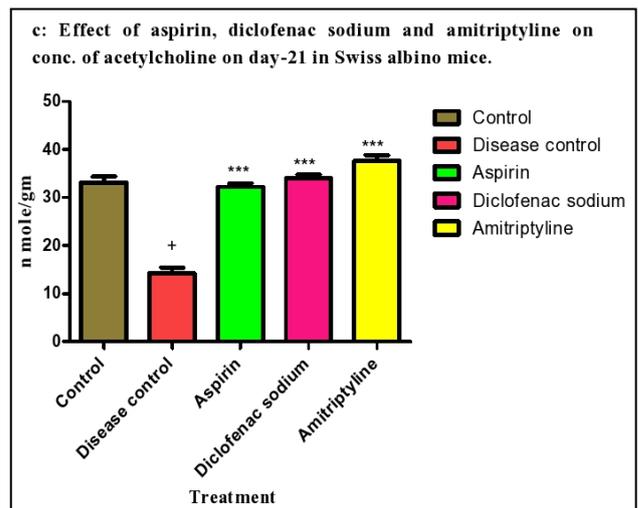
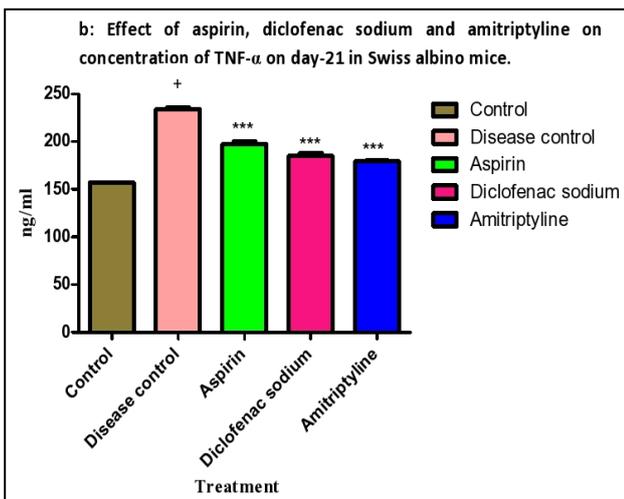
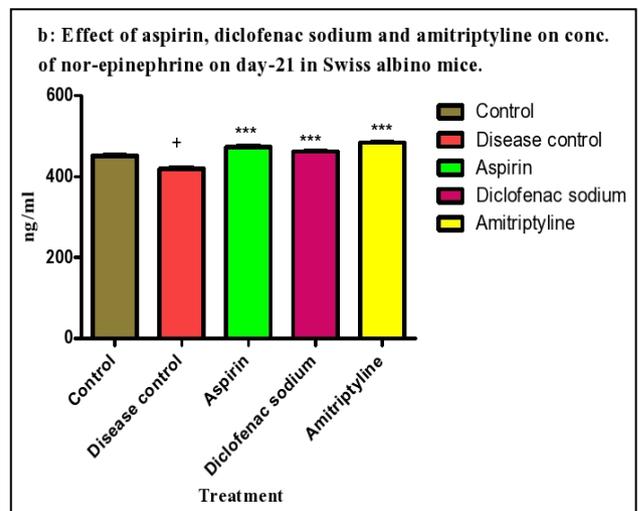
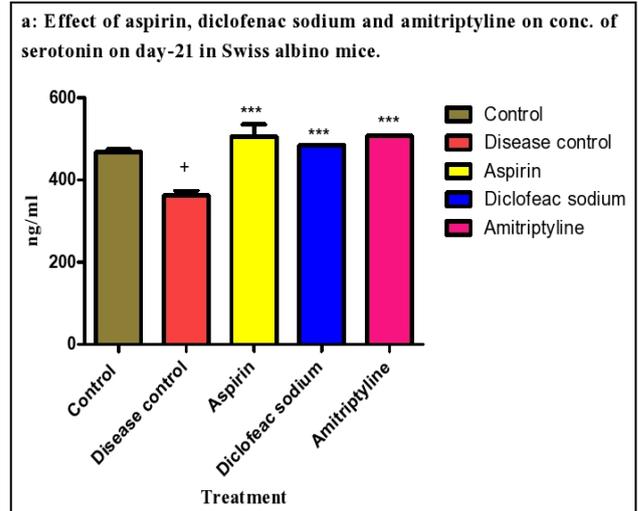
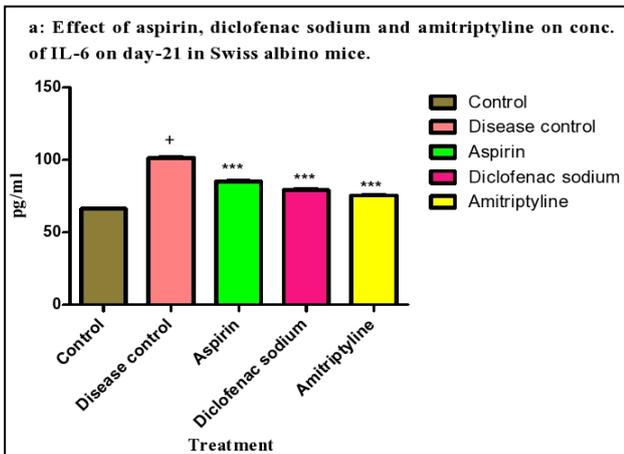


Figure 2: Effect of aspirin, diclofenac sodium and amitriptyline on a) conc. of IL-6 and on b) conc. of TNF-α on day-21 in Swiss albino mice. N=10, Values expressed as Mean±SEM, one-way ANOVA, followed by post hoc Dunnett’s test, *p<0.001 V/S disease control and +p<0.01 V/S control group.**

Figure 3: Effect of aspirin, diclofenac sodium and amitriptyline on a) conc. of serotonin b) conc. of nor-epinephrine c) conc. of acetylcholine on day-21 in Swiss albino mice. N=10, Values expressed as Mean ±SEM, one-way ANOVA, followed by post hoc Dunnett’s test, *p<0.001 V/S disease control and +p<0.01 V/S control group.**

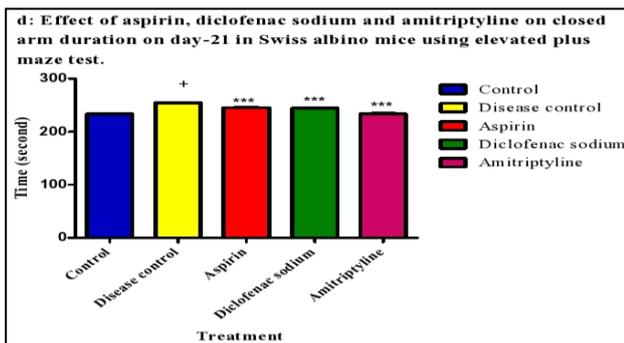
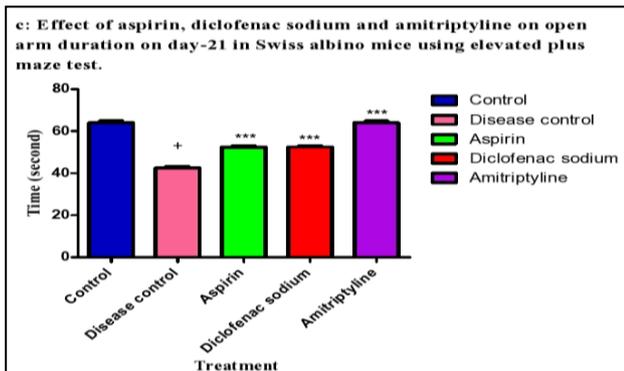
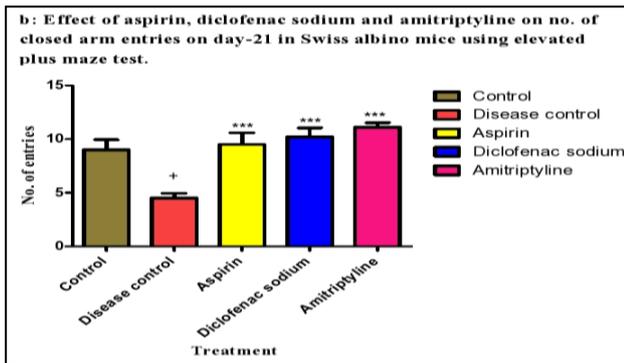
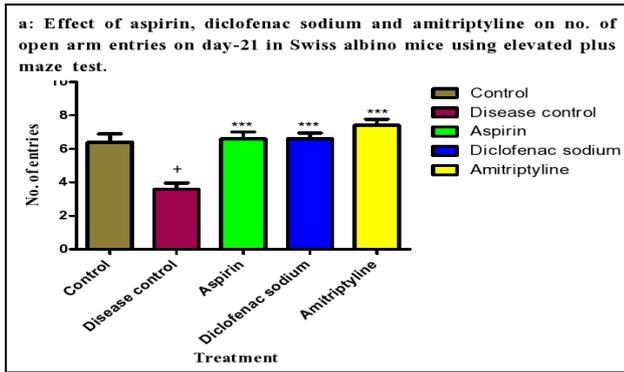


Figure 4: Effect of aspirin, diclofenac sodium and amitriptyline on a) no. of open arm entries b) no. of closed arm entries c) open arm duration and d) closed arm duration on day-21 in Swiss albino mice by using elevated plus maze test. N=10, Values expressed as Mean ±SEM, one-way ANOVA, followed by post hoc Dunnett’s test, *p<0.001 V/S disease control and +p<0.01 V/S control group.**

Tail suspension test

In the tail suspension test immobility time was decreased significantly in the aspirin, diclofenac sod. treated groups (Table 2) on day 21 when compared with the disease control group and was comparable with the standard drug amitriptyline treated animals.

Table 1: Effect of Aspirin, Diclofenac Sodium and Amitriptyline on immobility time on day-21 in swiss albino mice using tail suspension test.

S. no.	Group	Treatment	Immobility time (sec.)
1.	Control	Saline solution 1ml	204.1±0.8090
2.	Disease control	Formalin injection 0.1 of 2%	243.2±0.7860 ⁺
3.	Aspirin	14 mg/kg dissolved in warm water	204.5±1.067 ^{***}
4.	Diclofenac sodium	10 mg/kg dissolved in dis. Water	204.8±0.9165 ^{***}
5.	Amitriptyline	10 mg/kg dissolved in dis. Water	211.5±0.9574 ^{***}

N=10, Values expressed as Mean ±SEM, one-way ANOVA, followed by post hoc Dunnett’s test, ***p<0.001 V/S disease control and +p<0.01 V/S control group.

DISCUSSION

Many studies proved that there is association between inflammatory mediators and central neurotransmitters. On this previous basis, present study was taken up to prove antidepressant effect of anti-inflammatory drugs such as aspirin and diclofenac sodium.

In study one, light dark box test was used for screening locomotor activity in animals. Latency of first crossing decreased, no. of crossing increased and time spent in dark area decreased significantly in the aspirin, diclofenac sod. treated animals when compared with the disease control group of animals. Aspirin, diclofenac sod. treated animals showed statistically significant effects comparable with that of standard amitriptyline treated animals.

In study two, forced swim test was used for screening antidepressant activity. The immobility time was decreased significantly in the aspirin, diclofenac sod. treated group of animals when compared with the disease control animals and showed statistically significant result when compared with the standard drug amitriptyline treated animals.

In study three, estimation of inflammatory mediators was done from blood samples of swiss albino mice treated

with aspirin, diclofenac sod. and amitriptyline. The concentration of IL-6 and TNF- α were decreased significantly in the aspirin, diclofenac sod. treated group of animals when compared with the disease control group of animals.

In study four, estimation of neurotransmitters was done from brain homogenate of swiss albino mice treated with aspirin, diclofenac sod. and amitriptyline. The concentration of serotonin, nor epinephrine and acetylcholine increased significantly in aspirin, diclofenac sod. treated group of animals when compared with the disease control group of animals.

According to study 3rd and 4th the concentration of inflammatory mediators decreased and concentration of central neurotransmitters increased in depressed swiss albino mice treated with aspirin and diclofenac sod. As a non-steroidal anti-inflammatory drug (NSAID'S) aspirin and diclofenac sod. inhibits cyclooxygenase-1(COX-1) and cyclooxygenase-2 (COX-2), inhibition directly affects the central nervous system's serotonergic system. These drugs increase concentration of serotonin in both temporal and frontal parietal cortices. So, these drugs can treat depression caused by reduced level of serotonin.

In study five, elevated plus maze test was used. No. of open arm entries increased, no. of closed arm entries increased, time spent in open arm increased and time spent in closed arm decreased significantly in aspirin, diclofenac sod. treated group of animals when compared with disease control group of animals.

In study six, tail suspension test was used for screening antidepressant activity. The immobility time was decreased significantly in aspirin, diclofenac sod. treated group of animals. When compared with disease control group of animals and statistically significant results were come when compared with standard drug amitriptyline treated group of animals.

Response of fibroblast to cholinergic stimulation through nicotinic-acetylcholine receptor subunit inhibits production of inflammatory cytokines. So, acetylcholine decreases TNF- α and IL-6 release consequently.²⁴

Increased level of inflammatory cytokines produced changes in neurotransmitters level as well as function, which results into neuropsychiatric diseases and depression.^{10,25} Adrian found that IL-6 decreased the nor-epinephrine level, IL-6 β decreased production of acetylcholine.^{8,26} Proinflammatory cytokines have role in the pathophysiology of depression. TNF- α increases the risk of depression.²⁷ Saba Khan et al (2005) found the potential antidepressant, anti-stress and anxiolytic activity of aspirin in rodents.²⁸ Inflammatory cytokines produced symptoms of major depressive disorder.²⁹ Andrew et al found the role of cytokines in the pathophysiology of major depression and reported that depressed patients have an increased level of cytokines.³⁰ Inflammatory

cytokines effects on neuroendocrine processes. Cytokines plays an important role in development of depression. IFN- α decreases the level of serotonin and therapeutic administration of IFN- α produced depressive symptom, IL-1 and IL-6 contributes to the pathophysiology of depression.^{31,32} Michael Maes proved that serum IL-1 level was increased in depressed animals in compared with controlled animals.³³ Alexa Kabiersch et al found that systemic administration of IL-1 altered the metabolism of nor epinephrine, which is an important neurotransmitter that controls the neuroendocrine function by the central nervous system. They also proved that IL-6 altered the 5-HT metabolism in hippocampus.^{34,35} Szelenyi et al proved that TNF- α inhibited release or production of nor epinephrine.³⁶

Our study results and previous work done on this subject proved that anti-inflammatory drugs, aspirin and diclofenac sod. have antidepressant activity.

CONCLUSION

From this study, we can conclude that Our study results and previous work done on this subject proved that anti-inflammatory drugs, aspirin and diclofenac sodium have antidepressant activity.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethical Committee, Visveswarapura Institute of Pharmaceutical Sciences, Bengaluru. (IAEC No: 152/PO/ReBi/S/99/CPCSEA).

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