

# BEHAVIORAL AND BIOCHEMICAL EFFECTS OF *CANNABIS SATIVA* AND THEIR MODULATION BY ANTIDEPRESSANT DRUGS

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## ABSTRACT

We aimed to study the effect of *Cannabis sativa* on brain oxidative stress and determine whether behavioral responses caused by cannabis could be reversed by standard antidepressant drugs. *Cannabis sativa* (5, 10 or 15 mg/kg) (expressed as  $\Delta^9$ -tetrahydrocannabinol) was given alone or with fluoxetine, sertraline or imipramine, once daily subcutaneously (s.c.) for 24 days. In the forced-swimming test, the immobility time, was significantly increased in mice treated with cannabis (5-15 mg/kg, s.c.) starting from the 9<sup>th</sup> day post-injection. Fluoxetine (20 mg/kg, s.c.) co-administered with cannabis (5 mg/kg, s.c.) resulted in significant decrease in the immobility time by the day 21 of the study compared with the cannabis only group. Mice co-administered sertraline or imipramine with cannabis were not statistically different from the vehicle control group as regards their immobility time. Cannabis resulted in a significant decrease in the rearing activity which was ameliorated by either fluoxetine or sertraline. *Cannabis sativa* increased brain reduced glutathione, but decreased the level of nitric oxide. Fluoxetine, sertraline or imipramine given with cannabis decreased malondialdehyde and increased reduced glutathione. In conclusion: The administration of cannabis decreases brain oxidative stress but exerts depressive-like effect and decreases rearing activity which can be reversed by antidepressant drugs. [www.relaquim.com](http://www.relaquim.com)

**Keywords:** Cannabis; antidepressants; brain oxidative stress

## RESUMEN

Nos propusimos estudiar el efecto de *Cannabis sativa* sobre el estrés oxidativo en cerebro y determinar si las respuestas causadas por cannabis sobre el comportamiento podrían revertirse por fármacos antidepresivos estándar. *Cannabis sativa* (5, 10 o 15 mg/kg) (expresada como  $\Delta^9$ -tetrahidrocannabinol) se administró subcutáneamente (s.c.) sola o con fluoxetina, sertralina o imipramina una vez al día por 24 días. En la prueba de nado forzado, el tiempo de inmovilidad, se incrementó significativamente en los ratones tratados con cannabis (5-15 mg/kg, s.c.) a partir del 9<sup>o</sup> día post-administración. Fluoxetina (20mg/kg, s.c.), coadministrada con cannabis (5 mg/kg, s.c.), dio como resultado una disminución significativa en el tiempo de

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inmovilidad alrededor del día 21 del estudio comparado con el grupo tratado sólo con cannabis. Los ratones co-administrados con sertralina o imipramina y cannabis no fueron estadísticamente diferentes del grupo control de vehículo respecto al tiempo de inmovilidad. Cannabis provocó una disminución significativa en la actividad de levantamientos que fue aumentada tanto por fluoxetina como por sertralina. *Cannabis sativa* incrementó la glutatióna reducida en cerebro y disminuyó los niveles de óxido nítrico. Fluoxetina, sertralina o imipramina administrados con cannabis disminuyó la malondialdehído e incrementó la glutatióna reducida. En conclusión: La administración de cannabis disminuye el estrés oxidativo cerebral pero ejerce un efecto parecido a la depresión y disminuye la actividad de levantamientos que puede revertirse por fármacos antidepresivos. [www.relaquim.com](http://www.relaquim.com)

**Palabras clave:** Cannabis; antidepresivos; estrés oxidativo en cerebro

## INTRODUCTION

The cannabis preparations marijuana and hashish are the most common illicit drugs worldwide. These are derived from the female plant of *Cannabis sativa* L (family *Cannabaceae*). Marijuana is prepared from the dried flowering tops and leaves; hashish consists of dried cannabis resin and compressed flowers. Marijuana and hashish are usually smoked but may be also eaten or used in a tea form (Ashton, 2001). The administration of cannabis in man produces a spectrum of psychoactive effects including euphoria and relaxation, perceptual alterations, time distortion, and the intensification of ordinary sensory experiences, such as eating, watching films, and listening to music. Short-term memory and attention, motor skills, reaction time, and skilled activities are impaired while a person is intoxicated (Hall *et al.*, 1994). There is also elevated fatigue, drowsiness, dizziness and even severe transient psychotic symptoms with cannabis consumption (Kaufmann *et al.*, 2010). Nevertheless, cannabis is usually self-administered for its mood-altering properties, and has been described as an addictive, dependence-producing drug due to the production of euphoria, the presence of reversible psychological impairment, an abstinence syndrome, and tolerance. A mix-

ture of depressant and stimulant effects is noted at low doses; cannabis acts as a CNS depressant at high doses (Huestis, 2002). These psychomotor effects of cannabis are mainly attributed to the  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), the major psychoactive constituent in *Cannabis sativa* plant by acting on the cannabinoid (CB1) receptor that is highly expressed in the basal ganglia, the cerebral cortex and the cerebellum (Ameri, 1999; Pertwee, 1997, 2005; Svizenska *et al.*, 2008). Cannabinoid receptors are also activated by endogenous ligands, the endocannabinoids, a family of endogenous arachidonic acid derivatives, including N-arachidonyl ethanolamide (anandamide) and the 2-arachidonoylglycerol which is synthesized by the cell membrane (Sugiura *et al.*, 2002).

*Cannabis sativa* is being prescribed and used in several medical conditions. It is used in the management of chemotherapy-induced nausea and vomiting among cancer patients (Machado Rocha *et al.*, 2008) and for relief of spasticity in multiple sclerosis patients (Sastre-Garriga *et al.*, 2011). Medical cannabis is also used to alleviate chronic pain and arthritis (Swift *et al.*, 2005) and neuropathic pain caused by diabetes (Selvarajah *et al.*, 2010) and to improve the wellbeing in patients with depression (Denson and Earleywine, 2006).

Several studies have suggested a potential antidepressant effect for cannabis, its constituents (El-Alfy *et al.*, 2010; Zanelati *et al.*, 2010) or CB1 receptor agonists (Bambico *et al.*, 2007). Basal serum concentrations of the endocannabinoid ligands N-arachidonyl ethanolamide and 2-arachidonoylglycerol were significantly reduced in women with major depression relative to matched controls, indicating a deficit in peripheral endocannabinoid activity (Hill *et al.*, 2009). Meanwhile, the intake of cannabis has itself been associated with increased prevalence of depressive disorders (Bovasso, 2001). Cannabis-dependent subjects consumed greater amounts of cannabis, alcohol, and a variety of other drugs. They also had lower levels of motivation, happiness, and satisfaction with life, with higher levels of depression (Looby and Earleywine, 2007).

In view of these reported differences in effects of *Cannabis sativa* as regards depression, the aim of the study was to: (1) examine the effect of cannabis in the forced-swim test (a test for depressive-like behavior) (Porsolt *et al.*, 1978) in normal mice and after treatment with the selective serotonin reuptake inhibitors (SSRIs) fluoxetine and sertraline as well as the tricyclic drug imipramine with view to a possible modulatory effect of antidepressant drugs; (2) observe the effect of cannabis on exploratory behavior (rearing); (3) evaluate the effect of cannabis administration on antioxidant reserve and oxidative stress markers reduced glutathione (GSH), malondialdehyde (MDA) and nitric oxide in brain under these experimental paradigms since people who suffer from depression have abnormal levels of oxidative stress (Yager *et al.*, 2010; Sarandol *et al.*, 2007). A total extract from *Cannabis sativa* was used based on the fact that the effect of the whole plant which is abused by humans differs from that of THC in view of its content of other cannabinoids, terpenoids and flavonoids (Russo and McPartland, 2003).

## MATERIALS AND METHODS

### Animals

Swiss male albino mice 25-30 g of body weight were used. Standard laboratory food and water were provided *ad libitum*. Mice were obtained from animal house colony of the National Research Centre, Cairo. All animals were acclimatized to the laboratory conditions for 7 days before the beginning of the experiments. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

### Drugs and chemicals

*Cannabis sativa* L. plant was supplied by the Ministry of Justice- Egypt. Fluoxetine hydrochloride (Amoun Pharmaceutical Co., Cairo, A.R.E.), sertraline hydrochloride (Pfizer Egypt, Cairo, A.R.E.) and imipramine hydrochloride (Novartis Pharma, Cairo, A.R.E.) were used and dissolved in isotonic (0.9% NaCl) saline solution immediately before use.

### Preparation of *Cannabis sativa* extract

*Cannabis sativa* extract was prepared from the dried flowering tops and leaves of the plant. The method of extraction followed that described by Turner and Mahlberg (1984) with modification. In brief, 10 g of dried cannabis was ground with a mortar and pestle. Decarboxylation of the plant material was achieved by placing the sample in a glass test tube (30 mL) and covering it with aluminum foil. The test tubes were placed in boiling water bath (100 °C) for 2 h. Ten milliliters of analytical grade chloroform was added and allowed to react for 1 h. The dried cannabis was extracted three times and fractions were combined, filtered over filter paper and collected in a 100 mL volumetric flask. The filtrate was

evaporated under a gentle stream of nitrogen (on ice and protected from light and stored at 4 °C) and protected from light in an aluminum-covered container, which provided the dry extract as residue.

The residue (dry extract) is suspended in 2 mL of 96% ethanol and the total volume in the volumetric flask increased to 100 mL by adding distilled water. The extract was injected s.c. at doses of 5, 10 and 15 mg/kg (expressed as  $\Delta^9$ -THC). The injection volume was 0.3 ml/mouse. Tetrahydrocannabinol (THC) content was quantified using "GC mass spec". The dry extract contained 10% of the  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC).

Cannabinoids are enzymatically biosynthesised in the plant as their corresponding carboxylic acid forms (Taura *et al.*, 2007). Neutral cannabinoids are formed *via* decarboxylation (loss of CO<sub>2</sub>) of the acidic cannabinoids during exposure to light, heat (e.g. smoking), or as a result of prolonged storage (Thakur *et al.*, 2005) (Fig. 1).

The decarboxylation is carried out by heat in water bath at 100 °C for time. The <sup>1</sup>H-NMR proves that there is no signal corresponding to the carboxylic acid (COOH) around 12-13 ppm (Fig. 2).

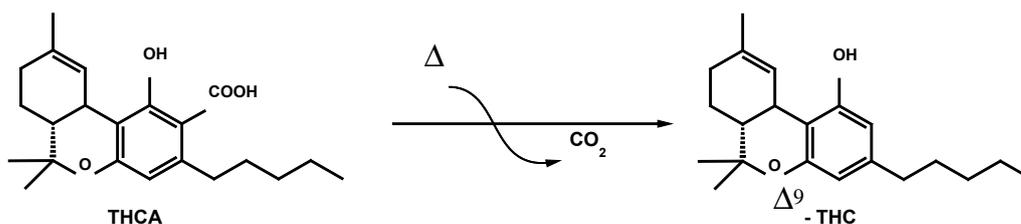
## STUDY DESIGN

### Experiment 1: Effect of cannabis alone

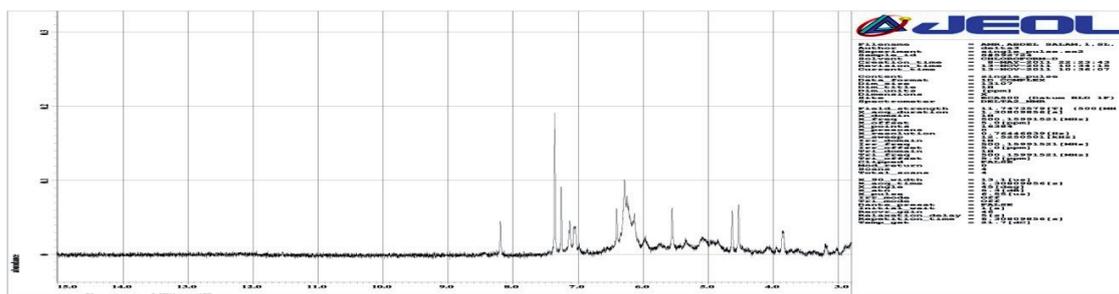
Twenty eight mice were randomly divided into four groups; each of 7 animals. Mice of the 1<sup>st</sup> (normal control) group were injected s.c. with an ethanol/distilled water (5 ml/kg). Animals of the 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> groups were injected s.c. with cannabis extract (5, 10, 15 mg/kg, respectively) daily for 24 days.

### Experiment 2: Effect of antidepressant drugs

Mice were treated with ethanol/distilled water (1<sup>st</sup>; normal control), fluoxetine (20 mg/kg) (2<sup>nd</sup> group), sertraline (20 mg/kg) (3<sup>rd</sup> group) or imipramine (20 mg/kg) (4<sup>th</sup>



**Fig. 1.** The decarboxylation of the acidic cannabinoids (tetrahydrocannabinolic acid: THCA) to delta 9-tetrahydrocannabinol ( $\Delta^9$ THC).



**Fig. 2.** <sup>1</sup>H-NMR of *Cannabis sativa* extract. No signal corresponding to the carboxylic acid (COOH) is observed around 12-13 ppm.

group) s.c. daily for 24 days (n = 7/group).

### Experiment 3: Effect of cannabis in combination with antidepressant drugs

Mice received ethanol/distilled water (1<sup>st</sup>; normal control), fluoxetine (20 mg/kg) + cannabis (5 mg/kg) (2<sup>nd</sup> group), sertraline (20 mg/kg) + cannabis (5 mg/kg) (3<sup>rd</sup> group) or imipramine (20 mg/kg) + cannabis (5 mg/kg) (4<sup>th</sup> group) s.c. daily for 24 days (n = 7/group).

In the three previously mentioned experiments, behavioral tests (rearing activity and forced swimming test) were done before the start of treatment (baseline), and then were examined twice weekly for 24 days. At the end of experimental period, mice were euthanized by decapitation under ether anesthesia. The brain of each mouse was excised and kept at -80 °C for estimation of reduced glutathione (GSH), lipid peroxidation (MDA) and nitric oxide determination in brain homogenate.

### Behavioral tests

All behavioral tests were conducted in quiet rooms 1h after drug injection.

### Forced-swimming test

This test was conducted according to the method of Porsolt *et al.* (1978). In brief, mice were placed individually in a glass cylinder (diameter 12 cm, height 24 cm) filled with water at a height of 12 cm, without the possibility of escaping. Water temperature was maintained at 25 ± 2°C. The animal was forced to swim for 6 min and the duration of immobility was measured. Mice were judged immobile when floating motionless or making only those movements necessary to keep its head above water. The duration of immobility was measured by an observer blind to the treatment conditions (Porsolt *et al.*, 1978) after treatment with vehicle, only cannabis (5, 10 or 15 mg/kg, s.c.), fluoxetine, sertraline, imipramine or after cannabis (5 mg/kg) co-administered with the antidepressant drugs. Water was chan-

ged after every swimming test to eliminate urine, excrement, and fur. After the swimming session, the mice were removed from the cylinder, dried with towels, and placed gently under a heating lamp for 15-30 min.

### Rearing activity

The open field was an acrylic round device (30 cm height) with a central circle (10 cm diameter). Mice were individually placed in the centre of the device. During the next 5 min the number of rears (R) (number of times seen standing on hind legs or on the wall) were recorded (Carbajal *et al.*, 2009).

## BIOCHEMICAL ANALYSIS

### Lipid peroxidation

The lipid peroxides content in brain homogenate was determined by monitoring the thiobarbituric acid reactive substance formation as described by Ruiz-Larea *et al.* (1994). In brief, 0.5 ml of the supernatant of brain homogenate was added to exactly 4.5 ml of working reagent (1 volume from 0.8% thiobarbituric acid + 3 volume from 20% trichloroacetic acid). The mixture was incubated for 20 minutes in boiling water bath then left to cool and centrifuge at 4000 rpm for 5 minutes. The pink color (malondialdehyde) was measured at 535 nm against blank (distilled water instead of sample). Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$  and the results were expressed as nmol MDA/g tissue.

### Reduced Glutathione

Determination of reduced glutathione content (GSH) in brain homogenate was determined according to the colorimetric method of Ellman (1959) modified by Bulaj *et al.* (1998). In brief, the brain was homogenized in phosphate buffered saline (pH 8.0) using a homogenizer. The homogenate

was then centrifuged at 4000 rpm/5 minutes at 4 °C in a cooling centrifuge then 0.5 ml of the supernatant was added to 0.5 ml of trichloro-acetic acid 10%. The mixture was vortex-mixed and centrifuged at 4000 rpm/5 min at 4 °C. In a clean test tube; 1.8 ml of phosphate buffer pH 8.0 was added to 0.1 ml of the supernatant and 0.1 ml of Ellman reagent. The absorbance was read at wave length 412 nm after exactly 5 minutes against blank (distilled water instead of sample). Reduced glutathione levels were calculated using the extinction coefficient of  $1.36 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ . The results were expressed in  $\mu\text{mol GSH/g tissue}$ .

### Nitric Oxide

Nitric oxide was determined in rat brain homogenate using a colorimetric method based on the Griess reaction according to the method of Miranda *et al.* (2001) with minor modification. Briefly, in Eppendorf tubes; zinc sulphate 30% was used for deproteinization of the samples (0.5 ml of supernatant of brain homogenate + 0.5 ml of  $\text{ZnSO}_4$ ); the mixture was centrifuged at 4000 rpm/15 minutes. In 96-well plate; 100  $\mu\text{l}$  of the obtained supernatants were placed in each well, followed by rapid addition of 50  $\mu\text{l}$  sulfanilamide (2% (w/v) in 5% (v/v) HCl) and 50  $\mu\text{l}$ : N-(1-naphthyl) ethylenediamine dihydrochloride (0.1% (w/v) in distilled water). The plate was then incubated at 37 °C for 45 minutes in a shaker water bath then cooled and the absorbance of the pink colored chromophore was measured at 540 nm using ELISA reader, against a blank treated in a similar manner to the test but using 100 $\mu\text{l}$  distilled water instead of the sample. A standard curve was constructed using serial dilutions ranging from 1.56-100  $\mu\text{M}$  of sodium nitrite ( $\text{NaNO}_2$ ) and was assayed using the previous method of nitrite determination. From the standard curve; an equation for calculation of nitrite concentration ( $\mu\text{M}$ ) was determined. The level of total nitrite/nitrate in the brain homogenate was expressed as  $\mu\text{mol/g tissue}$ .

### Statistical Analysis

Data are expressed as mean  $\pm$  SEM. The data were analyzed by one way ANOVA and by repeated measures ANOVA, followed by Dunnett's test for multiple group comparisons, using SPSS software (SAS Institute Inc., Cary, NC). A probability value of less than 0.05 was considered statistically significant.

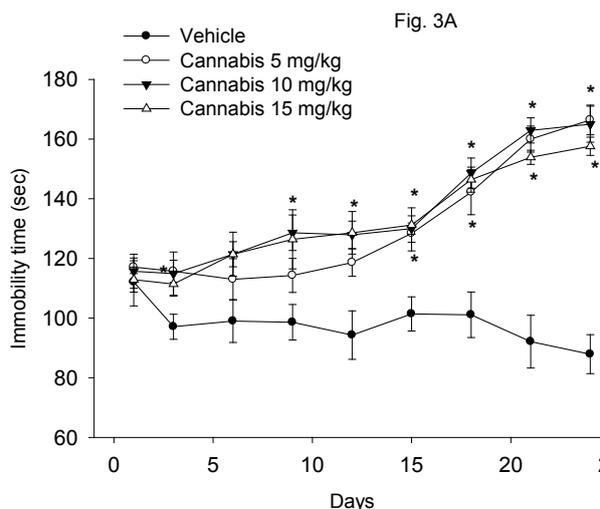
## RESULTS

### Behavioral tests

#### Forced-swimming test

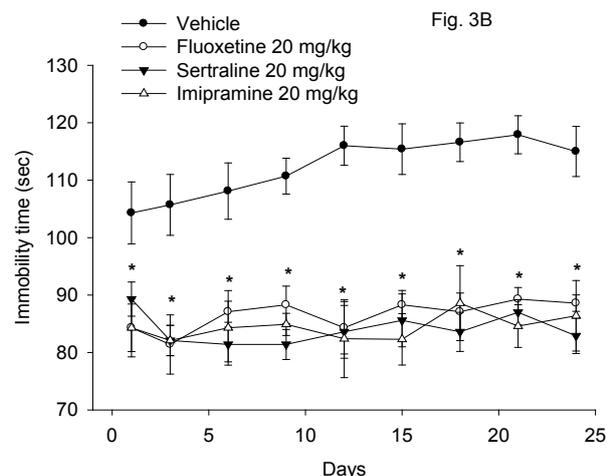
##### Effect of cannabis alone

The immobility time in the Porsolt's forced-swimming test after the repeated administration of cannabis at doses of 5, 10 or 15 mg/kg is shown in figure 3A. The floating time was significantly increased in mice treated with cannabis (5-15 mg/kg, s.c.) starting from the 9<sup>th</sup> day post-injection and afterwards. The immobility time increased 79.6, 87.7 and 89.3% at the end of the study by cannabis given at 5, 10 or 15 mg/kg, respectively. There was a significant drug effect ( $F_{3,24} = 42.76$ ;  $P = 0.001$ ), time effect ( $F_{8,192} = 24.81$ ;  $P = 0.001$ ) or treatment x time interaction ( $F_{24,192} = 5.75$ ;  $P = 0.001$ ).



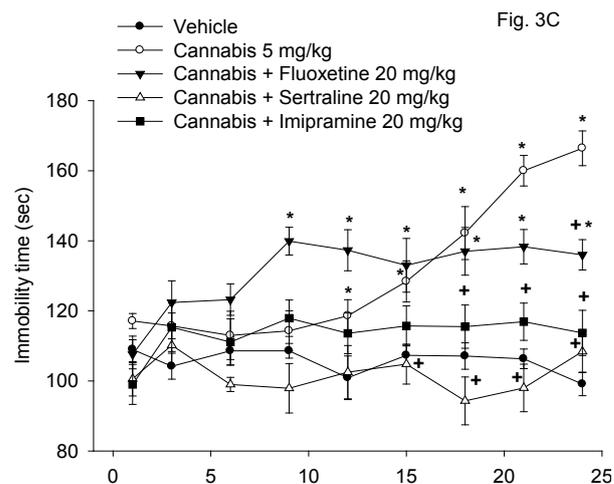
### Effect of antidepressant drugs

Treatment of mice with fluoxetine, sertraline or imipramine resulted in significant decrease in the immobility time compared to vehicle control group (Fig. 3B). There was a significant drug effect ( $F_{3,24} = 38.42$ ;  $P < 0.001$ ), but no significant time effect ( $F_{8,192} = 0.95$ ;  $P = 0.47$ ) or treatment x time interaction ( $F_{24,192} = 0.63$ ;  $P = 0.91$ ).



### Effect of cannabis in combination with antidepressant drugs

The immobility time in mice co-administered with fluoxetine (20 mg/kg, s.c.) and cannabis (5 mg/kg, s.c.) was significantly increased compared with the vehicle control group as well as with the cannabis only-treated group. By the day 21 of the study, however, the immobility time of the fluoxetine-cannabis group was significantly decreased compared with the cannabis only-treated group. Mice co-administered sertraline or imipramine with cannabis (20 mg/kg, s.c.) were not statistically different from the vehicle control group as regards their immobility time. Sertraline or imipramine significantly decreased the immobility time in cannabis-treated mice by the day 16 and 18 of the study, respectively. There was a significant drug effect ( $F_{4,30} = 22.02$ ;  $P < 0.001$ ), time effect ( $F_{8,240} = 7.58$ ;  $P = 0.001$ ) or treatment x time interaction ( $F_{32,240} = 4.59$ ;  $P = 0.001$ ) (Fig. 3C).

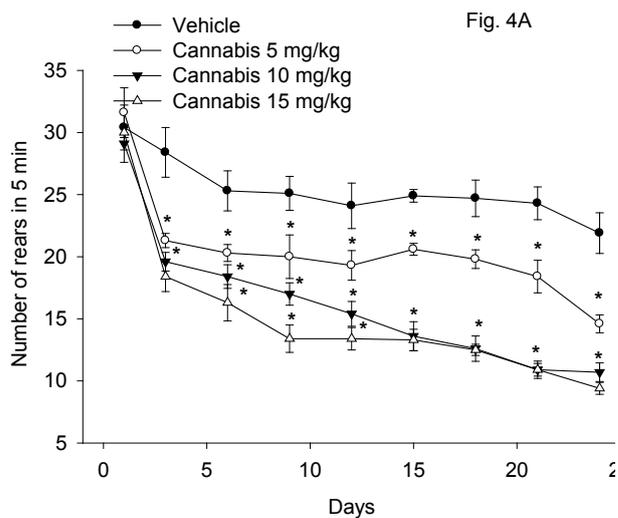


**Fig. 3A-C:** Effect of cannabis extract, antidepressant drugs or cannabis extract + antidepressants on immobility time in the forced swimming test in mice. \*:  $P < 0.05$  vs vehicle control group. +:  $P < 0.05$  vs only cannabis at 5 mg/kg.

### Rearing activity

#### Effect of cannabis alone

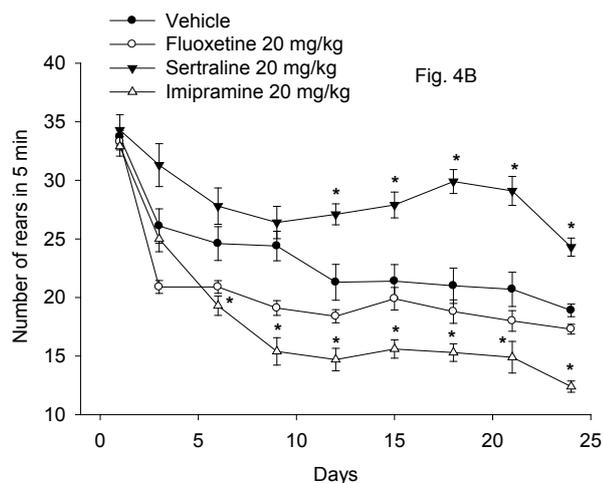
Figure 4A illustrates the effect of cannabis extract on rearing activity. Repeated measures ANOVA indicated a significant treatment effect:  $F_{3,24} = 64.23$ ;  $P < 0.001$ , significant time effect,  $F_{8,192} = 63.99$ ;  $P = 0.001$  and a significant treatment x time interaction,  $F_{24,192} = 2.97$ ;  $P = 0.001$ . The rearing activity was significantly decreased by cannabis compared to vehicle control group. This decrease in the rearing behavior was dose and time-dependant and



started 3 days after treatment with cannabis extract and continued throughout the study. The rearing activity decreased at the end of the study by 33.3, 51.1 and 58.9% after cannabis given at 5, 10 or 15 mg/kg, respectively.

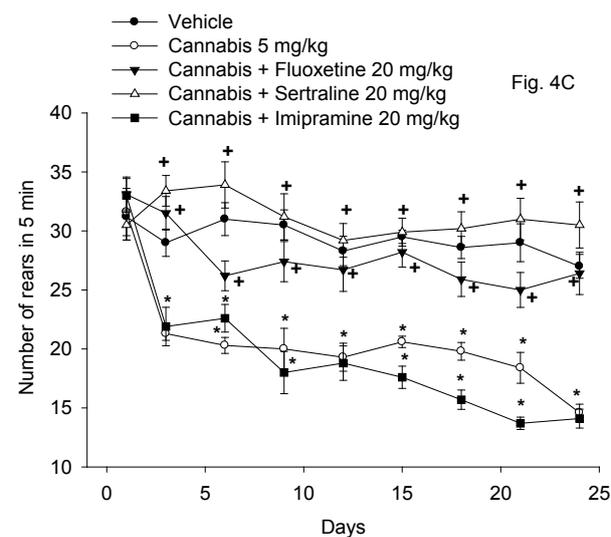
### Effect of antidepressant drugs

The results are shown in Fig. 4B. Repeated measures ANOVA indicated a significant treatment effect,  $F_{3,24} = 62.58$ ;  $P < 0.001$ , significant time effect,  $F_{8,192} = 55.03$ ;  $P = 0.001$  and significant treatment x time interaction,  $F_{24,192} = 3.02$ ;  $P = 0.001$ . The rearing activity in mice treated cannabis was markedly and significantly increased by sertraline or fluoxetine compared with the cannabis-only treatment group. The number of rears in mice given cannabis + fluoxetine showed no significant difference from the vehicle control group. Sertraline resulted in significant increase in number of rears in mice after 12 days of treatment till the 24<sup>th</sup> day (28.6% increase *vs.* vehicle control group,  $p < 0.05$ ). Meanwhile, the administration of imipramine to mice resulted in significant decrease in the rearing behavior after 6 days of treatment till the end of the study, as compared to the vehicle control group (34.4% decrease *vs.* vehicle control group,  $p < 0.05$ ).



### Effect of cannabis in combination with antidepressant drugs

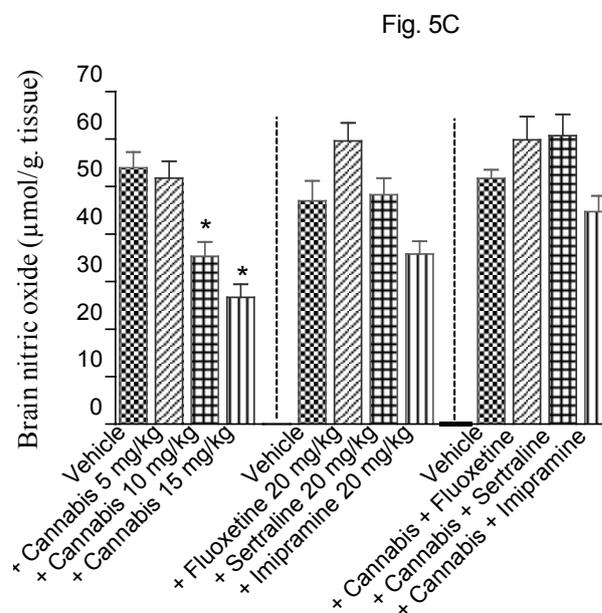
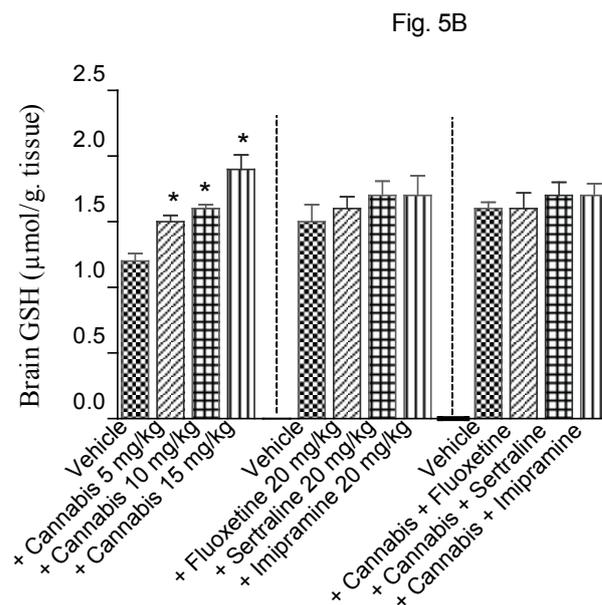
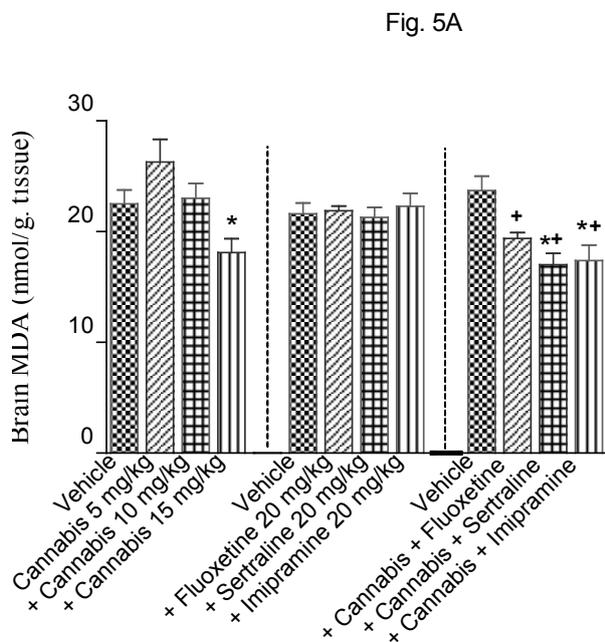
Figure 4C demonstrates the rearing activity in mice following cannabis treatment in combination with antidepressant drugs. Repeated measures ANOVA indicated a significant treatment effect,  $F_{4,30} = 82.72$ ;  $P < 0.001$ , significant time effect,  $F_{8,240} = 21.6$ ;  $P = 0.001$  and significant treatment x time interaction,  $F_{32,240} = 4.18$ ;  $P = 0.001$ . The administration of fluoxetine or sertraline at the dose of 20 mg/kg in combination with cannabis extract (5 mg/kg) resulted in no significant difference in the rearing activity of animals compared to vehicle control group. Mice treated with cannabis extract (5 mg/kg) and imipramine (20 mg/kg) showed a decrease in rearing activity compared with the vehicle treated group. The decrease in the rearing activity of mice that received imipramine and cannabis extract started after 6 days of administration till the end of the study (47.8% decrease *vs.* vehicle control group,  $p < 0.05$ ).



**Fig. 4A-C:** Effect of cannabis extract, antidepressant drugs or cannabis extract + antidepressants on rearing activity of mice. \*:  $P < 0.05$  *vs.* vehicle control group. + :  $P < 0.05$  *vs.* only cannabis at 5 mg/kg

### Biochemical studies

The administration of cannabis altered the redox status in brain with the effect being significant with the higher doses of the extract. Brain MDA was significantly decreased by the higher dose (15 mg/kg) of the extract (Fig. 5A). Brain GSH showed significant increase by 25, 3.3 and 58.3% after cannabis at 5, 10 or 15 mg/kg, respectively (Fig. 5B). Meanwhile, the administration of cannabis at 10 or 15 mg/kg resulted in a significant decrease in brain nitrite by 34.5 and 50.5% (Fig. 5C). Brain MDA or GSH were not significantly altered by fluoxetine, sertraline or imipramine alone (Figs. 5A and 5B). The level of nitric oxide, however, increased by 26.8% after fluoxetine treatment, but decreased by 23.8% following imipramine (Fig. 5C). There was a significant decrease in lipid peroxidation by 18.1, 28.3 and 26.6% after co-administration of cannabis with either fluoxetine, sertraline or imipramine, respectively when compared with the vehicle control group (Fig. 5A).



**Fig. 5A-C.** Effect of cannabis extract, antidepressant drugs or cannabis + antidepressant drugs on brain malondialdehyde (MDA), reduced glutathione (GSH) and nitric oxide (nitrite/nitrate). \*:  $P < 0.05$  vs corresponding vehicle control value. +:  $P < 0.05$  vs cannabis (5 mg/kg) only-treated group.

## DISCUSSION

This study investigated the effect of cannabis extract with known  $\Delta^9$ -THC content on the rearing activity and immobility in the forced swimming test in mice. The present findings indicate that the single and repeated daily administration of the extract at doses corresponding to 5, 10 and 15 mg  $\Delta^9$ -THC/kg resulted in a significant increase in immobility time. The administration of cannabis extract thus resulted in depressive-like effects. The increased immobility time was reversed by sertraline or imipramine, thereby, suggesting alteration of serotonergic and/or noradrenergic neurotransmission by cannabis. These results also indicate the ability of sertraline or imipramine to alleviate chronic stress due to repeated forced swimming. The SSRIs share the common property of inhibiting the reuptake of serotonin at synaptic terminals (Fuller, 1994). There is also an evidence suggesting inhibition of noradrenaline and dopamine reuptake by fluoxetine (Pozzi *et al.*, 1999; Bymaster *et al.*, 2002) and of dopamine reuptake by sertraline (Kitaichi *et al.*, 2010). The tricyclic drug imipramine, on the other hand, is a dual inhibitor of the reuptake of both noradrenaline and serotonin (Felton *et al.*, 2003).

Other researchers have shown that treatment with an endocannabinoid uptake inhibitor or CB1 receptor agonist induced comparable decreases in immobility in the forced swim test in rats (Hill and Gorzalka, 2005). In addition, the principal psychoactive component of marijuana  $\Delta^9$ -THC (2 and 6 mg/kg, i.p.) significantly prolonged the immobility time forced swim test; the effect being mediated by CB1 receptor- and 5-HT<sub>1A</sub> receptors (Egashira *et al.*, 2008). Studies also suggested that females may be more sensitive to the effects of THC than males. Adolescent female rats treated with (THC) for 11 days and left until adulthood presented significant 'behavioral despair' (forced swim test) paralleled by anhedonia

(sucrose preference). In contrast, male rats showed no behavioral despair but did present anhedonia (Rubino *et al.* 2008). Daily injections of THC (2 mg/kg) caused locomotor depression in both male and female rats dosed during early adolescence but only in female animals dosed during late adolescence (Harte *et al.*, 2010). Chronic (20 days) adolescent but not adult daily injection of CB (1) receptor agonist led to anxiety and depression-like behavior in the forced swim and sucrose preference test in rats. Serotonergic hypoactivity and noradrenergic hyperactivity were observed (Bambico *et al.*, 2010). It has been suggested that significant alterations in serotonergic systems may be rather related to acute activation of the endogenous cannabinoid system or to cannabis dependence accompanied by manifest depressive symptoms (Rose *et al.*, 2009). Studies in CB1-knockout on the other hand showed the opposite results. Thus, reduced exploration of the open arms of the plus-maze apparatus by CB1-knockout compared with wild-type animals was reported (Haller *et al.*, 2002). CB1 knockout mice also showed a higher sensitivity to exhibit depressive-like responses in the chronic unpredictable mild stress procedure suggesting an increased susceptibility to develop an anhedonic state (Martin *et al.*, 2002). In humans, inconsistent data exists as regards whether cannabis causes depression or not. In one survey, daily or once weekly adult users of cannabis reported less depressed mood and more positive affect than non-users (Denson and Earleywine, 2005). Other studies suggested that cannabis abuse was a risk factor for the development of depressive symptoms (Gregory and Bovasso, 2001) and that cannabis dependence was highly associated with independent depression (Dakwar *et al.*, 2011). Cannabis-dependent subjects had lower levels of motivation, happiness, and satisfaction with life, with higher levels of depression (Looby and Earleywine, 2007). Depressive disorders were

frequent in adolescents with substance (alcohol and cannabis) use disorder (Findling *et al.*, 2009) and the intake of cannabis has been associated with increased prevalence of depressive disorders (Bovasso, 2001), anxiety and mood disorders (Cheung *et al.*, 2010). Moreover, among patients with bipolar disorder, cannabis users exhibited less compliance and higher level of overall illness severity compared with non-users (van Rossum *et al.*, 2009).

The present findings also indicated that cannabis administration was associated with decreased rearing activity of mice. The latter is a frequently used measure of anxiety-like behavior (Henderson *et al.*, 2004); those with less exploratory activity (i.e. rearing) were considered more anxious (Bouwknicht *et al.*, 2007; Bogdanov *et al.*, 2012). It has been suggested that rearing is a useful marker of environmental novelty, that the hippocampal formation is a crucial component of the system controlling rearing in novel environments (Lever *et al.*, 2006). In other studies, decreased rearing has been taken as an indication of reduced anxiety (González-Trujano *et al.*, 2006). The results of the present study are in accordance with other studies showing decreased rearing activity after chronic THC treatment in rats (Miczek, 1976, 1979; Miczek and Dixit, 1980). The rearing activity in rats was also decreased by the endogenous endocannabinoid anandamide (Fride and Mechoulam, 1993). Studies also showed that THC strongly affects rearing activity more strongly than locomotion with tolerance being evident to the latter, but with no recovery of the reduced rearing activity when exposure to THC was continued for weeks (Miczek and Dixit, 1980; Miller and Drew, 1974). In the present study, the decrease in rearing activity was ameliorated by the SSRIs fluoxetine or sertraline, suggesting amelioration of the effect of cannabis on exploratory behavior by the antidepressant drugs.

Oxidative stress has been implicated in pathogenesis of depression and several

other brain disorders such as Parkinson's disease, Alzheimer's disease, bipolar disorder, major depression and schizophrenia (Sian *et al.*, 1994; Schulz *et al.*, 2000; Yao *et al.*, 2006; Lavoie *et al.*, 2011; Gawryluk *et al.*, 2011). In addition, there is evidence to support a benefit from increasing brain glutathione levels (e.g., *via* the glutathione precursor N-acetylcysteine) in conditions such as schizophrenia (Ng *et al.*, 2008; Duarte *et al.*, 2011). In the present study, the repeated administration of cannabis for 24 days was associated with significant and dose-dependent increase in brain GSH. The latter is a major antioxidant and plays an important role in the maintenance of the redox status of the cell and in protecting against oxidative damage by reactive oxygen species (Wang and Ballatori, 1998). Malondialdehyde, a marker of lipid peroxidation (Gutteridge, 1995) showed a significant decrease in brain by the highest dose of cannabis, suggesting an antioxidant effect for the extract. Moreover, the co-administration of fluoxetine, sertraline or imipramine with cannabis was associated with a significant decrease of brain MDA. Indeed, a neuroprotective effect of cannabis has been suggested. In rat cortical neuronal cultures,  $\Delta^9$ -THC and cannabidiol, a nonpsychoactive component of *Cannabis sativa*, exhibited antioxidant activity and reduced glutamate toxicity by cannabinoid receptor-independent mechanism (Hampson *et al.*, 1998).

In the present study, nitric oxide (the concentrations of nitrite/nitrate) is also decreased in the brain following cannabis injection. Lifetime major depressive disorder patients had lower total plasma nitrite/nitrate concentrations compared to healthy controls (Wagner *et al.*, 2011; Garcia *et al.*, 2011). Other studies, however, reported elevated plasma nitrate concentrations in depression (Suzuki *et al.*, 2001). In the present study; nitric oxide is increased in the brain by fluoxetine. In contrast, nitric oxide is decreased by imipramine. The co-

administration of fluoxetine or sertraline with cannabis was associated with near normal values of nitric oxide. In several studies antidepressant drugs have been shown to modulate nitric oxide release within the brain (Ha *et al.*, 2006; Liu *et al.*, 2011; Krass *et al.*, 2011). Studies also suggested the involvement of nitric oxide in depression and in the mood elevating action of antidepressant drugs. Thus pretreatment with L-arginine counteracted the antidepressant-like effect of imipramine, venlafaxine and bupropion (Krass *et al.*, 2011), while inhibition of nitric oxide levels within the hippocampus can induce antidepressant like effects (Joca and Guimarães, 2006). In those with lifetime major depressive disorder antidepressant medications were associated with higher NO in plasma (Wagner *et al.*, 2011). Meanwhile, the antidepressant action of imipramine and venlafaxine involves suppression of nitric oxide synthesis (Krass *et al.*, 2011). Rats exposed to chronic forced swimming test showed depressive-like behavior with alteration in platelet morphology, activity and platelet nitric oxide synthesis, and/or in 5-HT concentrations; these changes were prevented by fluoxetine (González-Trujano *et al.*, 2012).

The psychoactive effects of cannabis preparations are largely mediated by  $\Delta^9$ -THC (Gaoni and Mechoulam, 1964). The latter is the major cannabinoid found in marijuana and hashish, and constituted 10% of the extract used in the present work, making it the most likely candidate responsible for the effects of cannabis observed in the present work. *Cannabis sativa*, however, contains more than 600 different chemical compounds including over 70 different cannabinoids. In addition to  $\Delta^9$ -THC, other important cannabinoids include cannabidiol (CBD), cannabinol (CBN) and tetrahydrocannabinol (THCV). These can result in different effects from those of  $\Delta^9$ -THC alone since  $\Delta^9$ -THC, is a CB1 and CB2 receptor partial agonist

while  $\Delta^9$ -THCV, behaves either as a CB1 antagonist or, at higher doses, as a CB1 receptor agonist (Pertwee, 2008). Whilst  $\Delta^9$ -THC prolonged the immobility time forced swim test (Egashira *et al.*, 2008), other cannabinoids e.g., cannabidiol exhibited antidepressant-like effects (Zanelati *et al.*, 2010). The involvement of 5-HT<sub>1A</sub> receptors has been suggested. The chemistry of cannabis is thus a complex one and it is likely that the final effect of the extract will depend on the relative abundance of different cannabinoids, their interaction as well as interaction with other non-cannabinoid constituents.

## CONCLUSIONS

The administration of cannabis for 24 days in mice resulted in decreased brain oxidative stress but induced a significant increase in immobility in the forced-swimming test and a decrease in the rearing activity, suggesting a depressant- and anxiety-like effect. These latter effects were improved by antidepressant drugs.

## CONFLICTS OF INTEREST

There are no conflicts of interest.

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