

Effect of *Dacryodes edulis* Pulp Oil Treatment on Restraint Stress-Induced Behavioral and Biochemical Alterations in Mice

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ABSTRACT

Background: A stressful stimulus is a crucial determinant of health and disease. Antidepressants are used to manage stress and their related effects. The present study was designed to investigate the effect of *D. edulis* pulp oil in restraint stress-induced behavioral and biochemical alterations in mice.

Methods: Animals were immobilized for a period of 24 hr. *D. edulis* pulp oil (5 and 10 ml/kg) was administered 24 hr after the acute immobilized stress. Various behavioral tests parameters for anxiety (open field test, elevated plus Maze test), cognitive performance (Morris water maze) were assessed, followed by biochemical assessments (catalase, malondialdehyde level, nitrite and protein) subsequently.

Results: 14 days *D. edulis* treatment in a dose of 5 and 10 ml/kg significantly attenuated restraint stress-induced behavioral (improved locomotor activity, and antianxiety like effect) and oxidative damage as compared to control (restraint stress).

Conclusion: Present study showed important neuroprotective effect of *D. edulis* pulp oil against acute restraint stress induced behavioral and biochemical modifications.

Keywords: *Dacryodes edulis*; stress; behavioral; biochemical; mice.

INTRODUCTION

There is an increasing risk of mental disorders, such as acute stress disorder (ASD), post-traumatic stress disorder (PTSD) and depression among survivors who were trapped in rubble during earthquake. There are various neuropsychiatric problems such as anxiety, cognitive dysfunction and depression, are generally associated with stress. [1] So stress is a crucial determinant for maintenance of health or disease. [2,3] Stress induces changes in emotional behavior, anxiety like state [4] that are associated with oxidative damage such as free radical damage. [2,3] To date,

drug therapies available include tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs) or noradrenalin (ISRN), and several other antidepressants such as monoamine oxidase inhibitors – MAOIs. [5] Reactive oxygen species (ROS) may play a role in some neuropsychiatric disorders such as major depression. There is some evidence that the activation of immune-inflammatory process, increased monoamine catabolism, and abnormalities in lipids may cause overproduction of ROS, lipid peroxidation, and reduced antioxidant enzyme activities, and these processes may be related to pathophysiology of depression. [6] It know

that the central nervous system is especially vulnerable to free radical damage because of brain's high oxygen consumption, abundant lipid content and relative paucity of antioxidant enzymes. [7]

Dacryodes edulis (G. Don) HJ. Lam (Burseraceae), is a potent natural antioxidant and is currently receiving considerable attention in relation to neuropsychological disorders. Phytochemical studies have revealed the presence of various chemical compounds that would justify the medicinal indications of this plant. The fruits of *D. edulis* are rich in lipids, proteins, vitamins and antioxidants. Pharmacological studies have shown psychotropic properties of antidepressant and anxiolytic types. [8,9] Recent studies demonstrated that *D. edulis* oil administered to animals daily for 30 days showed the antidepressant-like effect in unpredictable chronic mild stress-induced depression model in mice, by improving the symptoms of depression in the behavioral tests of forced swimming and tail suspension test. [10] In depression, the symptomatology mainly comprises of cognitive deficits, apathy and anxiety along with other affective symptoms. Because *D. edulis* have earlier been proved to possess antianxiety and cognition-enhancing attributes in different animal models, the present study was undertaken to explore the possible role of this drug of plant origin on experimental restraint-induced behavioral and biochemical modification in mice.

MATERIAL AND METHODS

Preparation of the essential oil

The fruits were pitted and the pulp put to dry room temperature until needed. Fractions of 500g dried pulp fruit were submitted to hydrodistillation for approximately 3h. Oil extracting was dried by placing at 37°C until needed. The concentrated oil sample was used for experiments, in the dilution of 10 % in distilled water.

Animals

Adult male mice aged between 6 to 8 weeks, from the animal house of the Faculty of Health Sciences of the Marien Ngouabi University (Brazzaville), were used. They were raised in polypropylene cages, at an ambient temperature of 25 °C and a light/dark cycle of 12h/12, with free access to water and food. All experiments were conducted in compliance with Directive 2010/6106/EU, on the protection of laboratory animals. [11]

Procedure of the 24-hour restraint.

The protocol described by Chu et al [12] was used. Briefly, the mice was placed in a ventilated clear plastic tube (3cm in diameter and 10cm in length) and subjected for 24 hours restraint from 10:00 a.m. to 10:00 a.m. of the next day, kept in dark with a background noise by the air-conditioning vents. The holes (0.5 cm in diameter) in the head and along the sidewall of the tube enabled air flowing. Animals could move head and anterior limb, but the body and hindquarters were not able to move or turn around. During the restraint, the animals had no access to food and water. Once the restraint ended, mice were put back to their home cages immediately, with access to food and water.

Drug administration.

At the end of restraint stress protocol, animals were divided into 4 lots of 5 mice; distilled water (5 ml/kg), fluoxetine (FLU) 2,5 mg/kg, *D. edulis* oil (DEO), 5 and 10 ml/kg. All product were administrated orally 24 hours after the restraint stress and for 14 days. The treatment was followed by behavioral tests.

Behavioral studies

Open field test

Locomotor activity and exploratory behavior were evaluated using the open field test. The open field apparatus consists of a cubicle box made up of plywood and is of the dimension 60 cm × 40cm. All the walls are painted black except for the floor, which is divided into 16 squares by thick grey lines. Each of the animals was placed in a corner and in the next 10 min, was

observed for ambulation (number of squares crossed) and number of upright. [13-14]

Elevated plus-maze

Anxiety parameter was tested by the Elevated plus maze test. The apparatus was made of plywood and consisted of two opposed open arms (50 cm x 10 cm), two opposed enclosed arms with no roof (50 cm x 10 cm x 40 cm), and an open square (10 cm x 10 cm) in the center. The maze was elevated 50 cm above the floor and was monitored by a video camera from above. The animals were placed on the center of the maze, facing one of the open arms. The number of entries and the time spent in open arms during the next 5 min were analyzed from videotapes by trained observers as described by Verma and al, [14] An arm entry was defined when all four limbs of the rat were on the arm.

Cognitive studies

Morris water maze test

To evaluate spatial memory, mice were tested in Morris water maze which is a black circular pool of 150 cm diameter and 60 cm height, filled with 24 – 26 °C water to a depth of 40 cm. It is divided virtually into 4 quadrants. In the center of the southeast quadrant, a circular Plexiglas platform (10 cm in diameter) was located, hidden 2 cm below the surface of water. Before starting the experiments, each mice was handled daily for 3 days and then, in trial day, the animal were accustomed to the water maze for with and without a platform.

Mice were randomly released in one of the four quadrants of the pool while facing the wall of the tank. On each trial, the mice was allowed to swim until it found and remained on the platform for 20 s. If 60 s had passed and the animal had not found the platform, it was guided to the platform and allowed to stay on the platform for 20 s. During all these stages a camera was installed above the pool to detect the pathway of the rats which was then received by a computer and recorded. The time latency to reach the platform (experiment with platform) were compared among groups. In the retention phase (24 h later),

the platform was removed and a 60-s probe trial was conducted to examine how well the mice had learned the exact location of the platform. The time spent in the target quadrant was compared among groups. [15]

Biochemical studies

All the animals were sacrificed by decapitation on the same day immediately after behavioral assessments. The brains were removed, rinsed in isotonic saline and weighed. A 10% (w/v) tissue homogenates were prepared with 0.1 M phosphate buffer (pH 7.4). The post nuclear fractions were obtained by centrifugation of the homogenate at 12000 × g for 20 min at 4°C.

Protein estimation

The protein content was measured according to the method of lowry using bovine serum albumin as standard. [16]

Lipid peroxidation assay

The quantitative measurement of lipid peroxidation in the whole brain was assessed as per method of Wills. [17] The amount of malondialdehyde formed was measured by the reaction with thiobarbituric acid at 532 nm using Perkin Elmer lambda 20 spectrophotometer. The results were expressed as nmol of malondialdehyde per mg protein.

Nitrite estimation

Nitrite is the stable end product of nitric oxide (NO) in living system. Accumulation of nitrite was measured in cell free supernatants from brain homogenates by spectrophotometer assay based on Griess reagent 15 (1% sulphanilamide/0.1% naphthylethylenediamine dihydrochloride/2.5% phosphoric acid) and incubated at room temperature for 10 min to yield a chromophore. Absorbance was measured at 543 nm spectrophotometrically. The nitrite concentration was calculated from a standard curve using sodium nitrite as standard and expressed as micro molar nitrite per ml homogenate. [18]

Statistical analysis

Statistical analysis of data (using InStat 3.036) was performed by applying one-way analysis of variance (ANOVA) followed by

Tukey test. A $p < 0,05$ was considered statistically significant.

RESULTS

Behavioral studies

Open-field test. Results show that DEO (5 and 10 ml/kg) significantly increased ($p < 0,05$) the number of upright of stressed animals and the scare crossed in open-field test, after 14 days administration compared to control group (figure 1 and 2).

Elevated plus maze test. 14 days administration of DEO reduce significantly the symptom of anxiety induced by 24 hour immobilized stress compared to control group. Animals treated by DEO and FLU show an increase number of open arms entries and time spent in this ($p < 0,0001$) (figure 3 and 4).

Anxiolytic effect

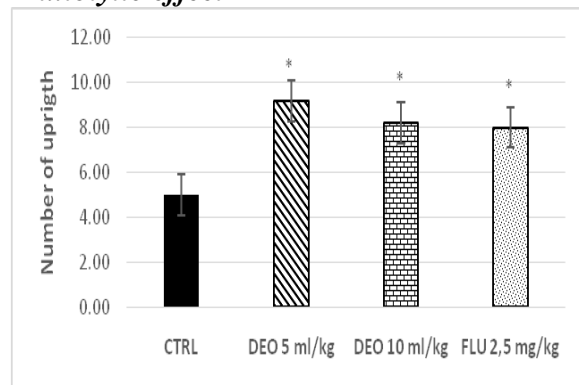


Figure 1: DEO effect on the number of upright of stressed animal in openfield test, after 14 days administration. CTRL: control group; DEO: *D. edulis* oil (5 and 10 ml/kg); FLU: fluoxetine (2,5 mg/kg). Data are expressed at mean ± SEM . (*): $p < 0,05$ compared to control (One-way ANOVA followed by Tukey's test), $n=5$.

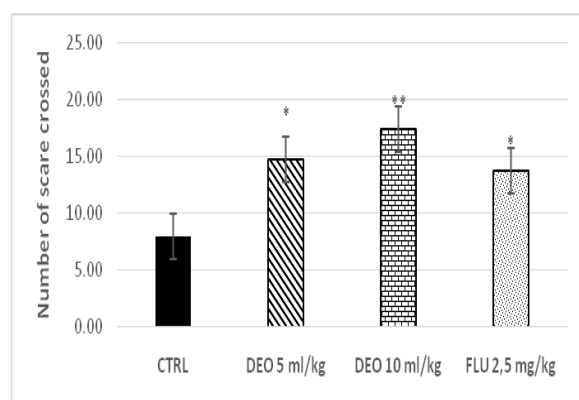


Figure 2: DEO effect on the number of scare crossed of stressed animals in openfield test, after 14 days administration. CTRL: control group; DEO: *D. edulis* oil (5 and 10 ml/kg); FLU: fluoxetine (2,5 mg/kg). Data are expressed at mean ± SEM. * : $p = 0,01$, ** : $p = 0,0004$ compared to control (One-way ANOVA followed by Tukey's test), $n=5$.

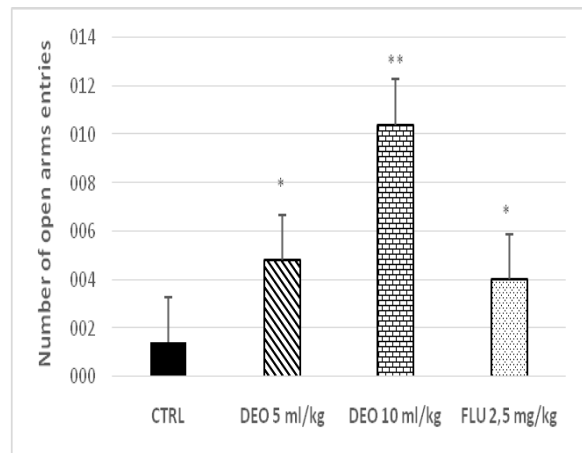


Figure 3: DEO effect on the number of open arms entries of stressed animals in the elevated plus maze test, after 14 days administration. CTRL: control group; DEO: *D. edulis* oil (5 and 10 ml/kg); FLU: fluoxetine (2,5 mg/kg). Data are expressed at mean ± SEM . *: $p = 0,001$; **: $p = 0,0007$ compared to control (One-way ANOVA followed by Tukey's test), $n=5$.

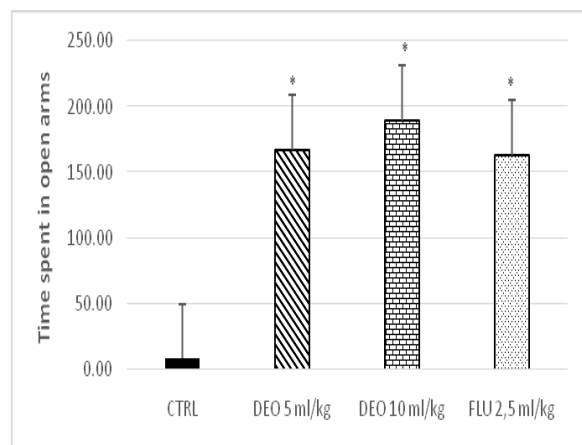


Figure 4: DEO effect on the time spent in open arms by stressed animals in the elevated plus maze test, after 14 days administration. CTRL: control group; DEO: *D. edulis* oil (5 and 10 ml/kg); FLU: fluoxetine (2,5 mg/kg). Data are expressed at mean ± SEM . *: $p < 0,0001$ compared to control (One-way ANOVA followed by Tukey's test), $n=5$.

Cognitive effect

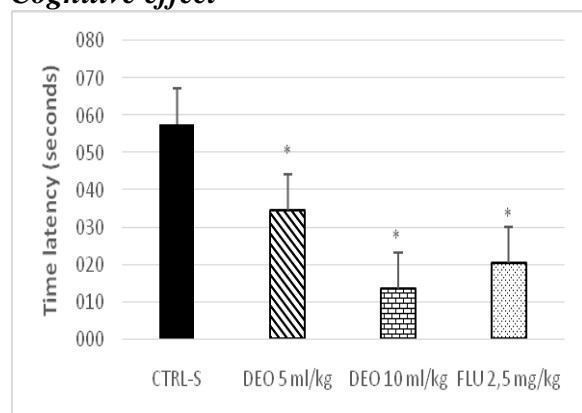


Figure 5: DEO effect on the time latency of stressed animals in MWM test, after 14 days administration. CTRL: control group; DEO: *D. edulis* oil (5 and 10 ml/kg); FLU: fluoxetine (2,5 mg/kg). Data are expressed at mean ± SEM . * : $p < 0,0001$ compared to control (One-way ANOVA followed by Tukey's test), $n=5$.

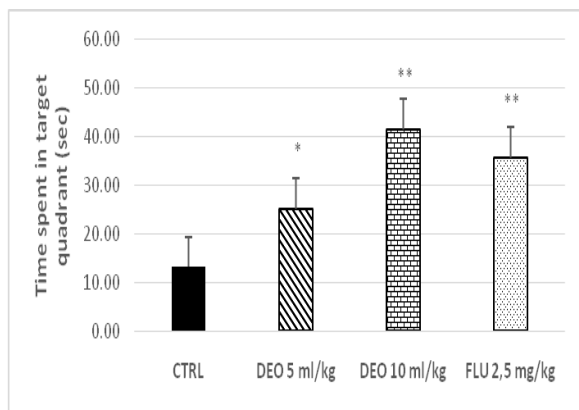


Figure 6: DEO effect on the time spent by stressed animals in target quadrant in MWM test, after 14 days administration. CTRL: control group; DEO: *D. edulis* oil (5 and 10 ml/kg); FLU: fluoxetine (2,5 mg/kg). Data are expressed at mean \pm SEM. (*): $p < 0,01$; (**): $p < 0,0001$ compared to control (One-way ANOVA followed by Tukey's test), $n=5$.

Cognitive studies

Morris water maze test. Treatment with DEO reduced significantly the latency to reach the platform in the MWM ($p < 0,0001$) when compared with the control group

Biochemical effect

Table I : DEO effect on biochemical parameters

Paramètres	Groups			
	CTRL	DEO 5 ml/kg	DEO 10 ml/kg	FLU 0,2 mg/kg
Proteins (g/l)	0,30 \pm 0,05	0,47 \pm 0,09*	0,58 \pm 0,14*	0,61 \pm 0,10*
Catalase	0,13 \pm 0,01	0,27 \pm 0,02**	0,31 \pm 0,06**	0,31 \pm 0,01**
MDA (n moles/mg pr)	0.633 \pm 0.04	0.452 \pm 0.04	0.283 \pm 0.06	0.229 \pm 0.01
Nitrites (μ mol/ml)	686.4 \pm 21.5	425.8 \pm 15.2*	341.6 \pm 19.4**	260.51 \pm 17.5**

DEO: *D. edulis* oil (5 and 10 ml/kg); FLU: fluoxetine (2,5 mg/kg). Data are expressed at mean \pm SEM . (*): $p < 0,05$; (**): $p < 0,001$ compared to control (One-way ANOVA followed by Tukey's test), $n=5$.

DISCUSSION

Stressful life events contribute to the development of many neurodegenerative and neuropsychiatric disorders including depression and anxiety. The ability of the biological system to cope stressful condition plays a crucial role in the body. [19] Many of these effects are thought to be mediated by stress induced neurochemical and hormonal abnormalities that are often associated with oxidative stress. Stress activates hypothalamus - pituitary - adrenal axis (HPA) axis and influences several neurological function at both central and peripheral level. Restraint stress has been reported to influence motor activity and anxiety like behavior, [20,21] and depression-like behaviors [20-22] in the animals.

In the present study, we investigated the neuroprotective effect of 14 days

(figure 5). Time spent in target quadrant was also increased by 14 days treatment of stressed animals (figure 6).

Biochemical studies

In this study, we have quantified the total protein in brain homogenate of stressed mice. Result show an increase of total protein in DEO and FLU group, with respectively $0,47 \pm 0,09$ ($p < 0,05$), $0,58 \pm 0,14$ ($p < 0,05$) and $0,61 \pm 0,10$ ($p < 0,05$). This increased of protein was correlate with the increase of catalase activity and MDA and nitrite levels. Values of catalase activity were $0,27 \pm 0,02$ ($p < 0,001$), $0,31 \pm 0,06$ ($p < 0,001$) and $0,31 \pm 0,01$ ($p < 0,001$), respectively with DEO (5 and 10 ml/kg) and FLU (2,5 mg/kg) versus control group ($0,13 \pm 0,01$). Table I present all of biochemical assessment.

administration of *D. edulis* on behavioral, cognitive and biochemical change induced by 24 hours restraint stress.

The open field test is conventionally designed to evaluate the state of anxiety. It's well documented by the previous research that in stressed animal, the motor activity will be decreased and inverse to this. The number of upright will be correspondingly impaired. [13] *D. edulis* treatment increased motor activity (number of squares crossed), such as fluoxetine. Marked behavioral changes might be due to alteration in the brain regions controlling motor activity and anxiety like behavior. Impaired motor activity could be due to stress induced depression.

The Elevated plus maze test depends on the fact that the anxiety-prone animals will prefer to stay in the dark closed arm of

the apparatus than in the open white arms. The number of open arms entries and time spent provide a measure of fear-induced inhibition of exploratory activity. These behavioral are reduced by anxiolytics and increased by anxiogenic agents. [23] In this study, *D. edulis* and fluoxetine administrated to stressed animals, showed significant increased number of open arms entries and time spent in the Elevated plus maze test. These findings corroborates with anxiolytic effect of *D. edulis* observed in the previous study. [9]

Morris water maze test incorporates three different parts of memory: reference memory, working memory and spatial learning. It demonstrated that MWM is an excellent model for testing the lesions associated with the hippocampal system of animals. [13]

The effects of DEO on learning and memory have also been suggested. DEO enhanced learning and memory abilities of the mice when examined using MWM. Indeed, *D. edulis* treatment for 14 days significantly improved latency time to find platform and time spent in target quadrant. Recently, we demonstrated that DEO exhibit benefic effect on memory and learning in naïf animal. [9]

Oxidative stress causes cellular damage and accelerates neuro-degeneration by inducing the reactive oxygen species (ROS) that oxidize vital cellular components such as lipids, proteins and DNA. [24;25] In the present study, *D. edulis* (5 and 10 ml/kg) treatment significantly attenuated lipid peroxidation, nitrite concentration and partially restored MDA, nitrite and catalase activity suggesting its antioxidant like effect. These findings are in agreement of clinical trial that also report elevated level of MDA in patients with affective disorders. [26] Likewise, other antidepressant such as fluoxetine has also been reported to reduce the MDA level in restraint animals. [26] Antidepressants drugs have also been reported to elevated antioxidant enzyme defense system particularly superoxide enzyme and catalase

activity. [27] Tsuboi et al reported an increased oxidative damage and weak antioxidant defense events are implicated in major depression. [25]

Present study further suggests DEO therapeutic potential against these stress related altered behavioral states.

In the present study, *D. edulis* treatment significantly produced analgesic effect. In the present study, 24-hr immobilized stress caused significantly oxidative damage as indicated by raise in lipid peroxidation, nitrite concentration and depletes reduced GSH and catalase activity. Several studies have shown the antioxidant activity of *D. edulis*. The leaves of the plant showed a significant antioxidant effect with different methods. [28-30] These results suggest that *D. edulis* could struggle damage caused by lipid peroxidation. [31,32] The antioxidant properties of *D. edulis* were confirmed by measurement of biochemical markers of oxidative stress (glutathione peroxidase, superoxide dismutase and selenium) in rats. Previous study showed a slight increase of these markers in rats fed with supplemented nutrition with oil of *D.edulis*. [33]

CONCLUSION

In summary, the present study has shown that DEO is effective in ameliorating immobilization stress-induced behavioral alterations and oxidative stress. The *D. edulis* neuroprotective effect may be related by the increasing antioxidant system. These findings further support the therapeutic potential of *D. edulis* pulp oil in the treatment of stress-related disorders.

Conflict of interest: authors declare there is no conflict of interest.

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