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Neuropharmacological Potential of Fragaria x ananassa Fruit Extracts: Attenuation of Anxiety-Like Behavior via Serotonergic Modulation and Catalase Upregulation.

Rajdeep Kaur*[1], Dr. Jyoti Gupta[2], Dr. Anjali Sharma[3]

[1]Research Scholar, IEC School of Pharmacy, IEC University, Baddi, Solan, Himachal Pradesh, 174103, India.
[2]Professor & Head, IEC School of Pharmacy, IEC University, Baddi, Solan, Himachal Pradesh, 174103, India.
[3]Associate Professor, Guru Gobind Singh College of Pharmacy, Yamunanagar, Haryana, 135001, India.
Corresponding author Rajdeep Kaur
Email: rajdeepdhaliwal90@gmail.com

ABSTRACT:

Background: Fragaria × ananassa has been known for its nutritional values and rich phytoconstituent composition. Its neuropharmacological properties, however, in terms of modulation of anxiety, brain monoamines and central oxidative stress remain poorly investigated.

Objective: To assess the chronic anti-anxiety effects, neurochemical changes and antioxidants mechanisms of the aqueous and n-hexane fruit extracts of Fragaria × ananassa on animal models.

Methods: The methods adopted were water extraction and n-hexane extraction of fruits. Qualitative phytochemical screening was performed. Swiss albino mice were divided into three groups, standard (Diazepam), control and extract treatment for both the extracts (50mg/kg, 100mg/kg p.o.) and treated for 21 days. Behavioral tests were performed on EPM, Light-Dark Box and OFT on days 0, 7, 14 and 21. The 5-Hydroxytryptamine (5-HT) levels and oxidative stress markers Malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) were determined in the brain tissue.

Results: The phytochemical screening revealed that aqueous and n-hexane fruit extracts of Fragaria × ananassa were rich in flavonoids, phenols, tannins, saponins, and alkaloids while FAH was rich in steroids and terpenoids. Both extracts (50 mg/kg and 100 mg/kg) significantly increased the number of entries into the open arm in EPM and the time spent in the light compartment in the Light-Dark test, and increased light-area entry and light occupancy in the Light-Dark test in a time-dependent manner. In the OFT, there was an increase in the number of line crossings over time without sedation. The concentrations of 5-HT in the brain increased significantly by aqueous and n-hexane extracts whereas Diazepam reduced the brain 5-HT level. In addition, aqueous and n-hexane fruit extracts decreased lipid peroxidation (MDA) and increased endogenous antioxidant defenses (GSH, SOD) and aqueous and n-hexane fruit extracts significantly increased Catalase activity in a dose-dependent manner.

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Conclusions: Chronic treatment with Fragaria x ananassa fruit extracts has significant anxiolytic-like effects, through serotonergic upregulation and central antioxidant preservation, with the aqueous extract showing a higher enzymatic antioxidant enhancement.

1. INTRODUCTION:

Anxiety disorders are one of the most common neuropsychiatric disorders in the world, impacting over 300 million people and accounting for a significant proportion of the world's disability and health care burden. These are disorders in which there is a chronic state of fear, excessive apprehension, autonomic hyperactivity, and behavioural abnormalities that significantly affect quality of life. Several drugs can be used to treat anxiety disorders, including benzodiazepines, serotonin-reuptake inhibitors (SSRIs) and serotonin–noradrenaline reuptake inhibitors (SNRIs); however, there are side-effects, such as tolerance, dependence, cognitive impairment, withdrawal, delayed onset of therapeutic effect, and others, associated with long-term use of these drugs (Bandelow et al., 2017; WHO, 2022). These restrictions have led to a growing demand to identify those natural bioactive compounds that are more safe for use and with multiple target pharmacological activities that can influence the complex pathophysiology of anxiety disorders (Lakhan & Vieira, 2010; Altemimi et al., 2017).

Medicinal plants have historically been a vital source of therapeutic compounds and several phytochemicals, like flavonoids, phenolic acids, tannins, alkaloids and terpenoids have been shown to have anxiolytic, antioxidant and neuroprotective effects in experimental models. Phytochemicals have pleiotropic effects by modulation of γ-aminobutyric acid (GABA), serotonergic neurotransmission, dopaminergic neurotransmission, suppression of oxidative stress, and suppression of neuroinflammatory responses, which are not observed with synthetic drugs, that alter a single molecular pathway (Salehi et al., 2020; Nabavi et al., 2015). In recent systematic reviews, medicinal plants have been identified as potential alternatives for the treatment of anxiety disorders due to their effects on several neurobiological pathways which have relatively less side effects (Mughal et al. 2022).

Fragaria × *ananassa* Duch. Garden strawberry (*Fragaria* ananassa, Rosaceae) is a fruit crop utilized throughout the world and known as a rich source of vitamins, dietary fibre and phenolic antioxidants. The presence of high levels of polyphenols and other secondary metabolites in *Fragaria* × ananassa makes them a valuable bi-product of agriculture, although their use in crops is not well-studied. The phytochemical composition of strawberry has been explored and found to contain many compounds with strong antioxidant, anti-inflammatory, antimicrobial and neuroprotective properties, such as quercetin and kaempferol glycosides, catechins, ellagitannins, ellagic acid derivatives, chlorogenic acid, caffeic acid derivatives, proanthocyanidins, and several triterpenoids (Kårlund et al., 2014; Kårlund et al., 2017; Giampieri et al., 2018).

Pharmacological activity of herbal extract largely depends on the solvent used in the extraction process because the extraction method depends on the polarity of the solvent which affects the type and concentration of the phytoconstituents recovered from plant materials. The hydrophilic compounds, such as phenolic acids, flavonoids, tannins and glycosides, are extracted in a higher proportion by polar solvents like water, while lipophilic compounds, which are non-polar, such as phytosterols, fatty acids, waxes, chlorophylls, and terpenoids, are extracted in a higher proportion by non-polar solvents like n-hexane. As such, extracts produced with solvents of varying polarities can often have very different chemical compositions and biological activity (Azwanida, 2015; Do et al., 2014). Hence, comparative assessment of aqueous and n-hexane extracts is a valuable approach for studying the connection between the solvent dependent phytochemical profile and therapeutic activity.

While a few studies have identified the phytochemical profile and antioxidant activity of *F.* × *ananassa* fruits and, although in less detail, detailed comparison between the aqueous and the n-hexane extract of the fruits with respect to their phytochemical profile and anxiolytic potential is still limited. Moreover, the contribution of the various phytoconstituents to the neuropharmacological activity of *F.* × *ananassa* is not fully understood. Hence, the present study was designed to comparatively assess the phytochemical composition and anxiolytic effects of the aqueous and n-hexane fruit extract of *Fragaria* × ananassa. The results of this research will be used as scientific evidence for the therapeutic potential of *Fragaria* × *ananassa* and it will help create plant-based remedies for anxiety disorders.

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2. MATERIALS AND METHODS

2.1 Plant Material

Fresh fruits of *Fragaria × ananassa* were collected from Kurali, Punjab, India and were taxonomically identified by the Department of Botany, Panjab University, Chandigarh, India.

2.2. Extraction

Fresh, fully ripe fruits of *Fragaria × ananassa* were collected, washed thoroughly with distilled water, and de-stemmed. The cleaned fruits were sliced uniformly and subjected to shade drying until a constant mass was achieved. The dried material was pulverized into a coarse powder using an electric grinder and sieved. Two distinct solvent fractions were prepared to isolate polar and non-polar phytoconstituents:

- **Aqueous Extract (FAA):** Prepared via extraction with double-distilled water to pull hydro-soluble components.
- **n-Hexane Extract (FAH):** Prepared using n-hexane to selectively isolate lipophilic fractions.

Both extracts were concentrated under reduced pressure, dried, and stored under refrigeration until experimental administration.

2.3 Preliminary Phytochemical Screening

The qualitative phytochemical screening of the aqueous and n-hexane extracts was carried out using standard phytochemical procedures described by Harborne (1998) and Evans (2009) respectively to detect the presence of major classes of secondary metabolites. The alkaloids, flavonoids and phenolic compounds, tannins, saponins, glycosides, carbohydrates, proteins, amino acids, steroids, terpenoids, fixed oils and reducing sugars were obtained in the extracts by methods of qualitative test. The intensity of the reactions was recorded as absent (−), weakly present (+), moderately present (++), or strongly present (+++).

2.4 Experimental animals

Swiss albino mice of 8 weeks old, weighing 20-25g, obtained from the Laboratory Animal center and housed in solid bottom polycarbonate cages under controlled environmental conditions (22 ± 2°C with 55 ± 5% humidity and a 12/12-hour light/dark cycle). Ethical clearance was obtained from the Institutional Animal Ethical Committee [IEC/SOP/IAEC/RP/2052-26/12]. All procedures were in strict compliance with relevant laws, the Animal Welfare Act, Public Health Services Policy and guidelines established by the Institutional Animal Care and Use Committee of the University.

2.5 Experimental Design

The experimental mice were divided into 6 groups, each consisting of 5 animals.

- **Group I (Vehicle Control):** Received normal saline.
- **Group II (Standard):** Received Diazepam as the positive reference standard (i.p.).
- **Group III (Aqueous Extract):** Received *Fragaria × ananassa* aqueous fruit extract (**FAA**, 50 mg/kg, p.o.).
- **Group IV (Aqueous Extract):** Received *Fragaria × ananassa* aqueous fruit extract (**FAA**, 100 mg/kg, p.o.).
- **Group V (n-Hexane Extract):** Received *Fragaria × ananassa* n-hexane fruit extract (**FAH**, 50 mg/kg, p.o.).
- **Group VI (n-Hexane Extract):** Received *Fragaria × ananassa* n-hexane fruit extract (**FAH**, 100 mg/kg, p.o.).

2.6 Evaluation of Anxiolytic Activity

2.6.1 Behavioural studies

Behavioural tests were performed on the 0^{th}, 7^{th}, 14^{th} and 21^{st} day of the study.

2.6.1.1 Elevated plus maze

Elevated plus maze model was as per the method of Lister, 1987. The plus-maze apparatus consisting of two open arms (38×5cm) and two closed arms (38×5×15cm) having an open roof, with the plus-maze elevated (50 cm) from the floor, was used to observe anxiolytic behaviour in animals.

Animals were fasted 18 h prior to the experiment. The dose administration schedule was so adjusted that each animal was having its turn on the elevated plus-maze apparatus 45 min after the administration of the dose. Each animal was placed at the centre of the elevated plus-maze with its head facing the open arms. During this 5 min experiment, the behaviour of the animals was recorded as the following parameters:

(a) Number of entries into the open arms

(b) Number of entries into the close arms

(c) Time spent by animal in open arms

(d) Time spent by animal in close arms

2.6.1.2 Open-field test

Open field test was as per the method of Archer, 1973**.** To evaluate the exploratory activities, animals were subjected to an open-field activity test. Animals were placed in an open field box (40 × 100 x 100 cm) marked off into 25 equal squares. All experiments were conducted in a quiet room under normal light. Each animal was tested in the apparatus once. To determine open field activity, each animal was placed for the first time in a fixed corner of the open field box. During a 5 minute period animals were permitted to move and explore the new environment (open field box).

During this period following parameters were recorded:

(a) The number of rows of crossed squares
(b) Number of grooming

A grooming episode was defined by repetitive movements of front paws or mouth.

2.6.1.3 Light and dark box model

Light and dark box model was as per the method of Costall *et al.,* 1989. The apparatus consisted of an open top wooden box and contains two distinct chambers, a black chamber (20×30×35cm) painted black and illuminated with dimmed red light and a bright chamber (30×30×35cm) painted white and brightly illuminated with 100 W light source which was located 17 cm above the box. The two chambers were connected through a small open doorway (7.5×5 cm) situated on the floor level at the centre of the partition. Animal was placed at the centre of brightly lit arena. Then following parameters were recorded:

(a) Number of entries into the bright arena
(b) Time spend in the bright arena

2.6.2. Assessment of Oxidative Stress in brain

2.6.2.1. Estimation of lipid peroxidation

The malondialdehyde content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid-reactive substances by the method of Wills (1966). Briefly, 0.1 ml of post mitochondrial supernatant and 0.1 ml of Tris–HCl were incubated at 37 °C for 2 h. After incubation 0.2 ml of 10% trichloro acetic acid was added and centrifuged at 1000×g for 10 min. To 0.3 ml of supernatant, 0.3 ml of 0.67% thiobarbituric acid was added and the tubes were kept in boiling water for 10 min. After cooling, 0.3 ml double distilled water was added and absorbance was measured at 532 nm. Thiobarbituric acid-reactive substances were quantified using an extinction coefficient of 1.56×105 M−1 cm−1 and expressed as nmol of malondialdehyde per mg protein.

2.6.2.2 Estimation of reduced glutathione

Reduced glutathione was assayed by the method of Jollow et al. (1974). Briefly, 0.05 ml of post-mitochondrial supernatant (10%) was precipitated with 0.05 ml of sulphosalicylic acid (4%). The samples were kept at 4°C for at least 1 h and then subjected to centrifugation at 1200×g for 15 min at 4 °C. The assay mixture contained 0.05 ml supernatant, 1.4 ml phosphate

buffer (0.1 M, pH 7.4) and 0.1 ml 5,5'- dithiobis (2-nitro benzoic acid) (Ellman's reagent, 0.1 mM, pH 8.0) in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm and the reduced GSH levels were expressed as µmoles/mg protein.

2.6.2.3 Estimation of superoxide dismutase

Cytosolic superoxide dismutase activity was assayed by the method of Kono (1978) The assay system consisted of 0.1 mM EDTA, 50 mM sodium carbonate and 96 mM of nitro blue tetrazolium (NBT). In the cuvette, 2 ml of above mixture was taken and to it 0.05 ml of post mitochondrial supernatant and 0.05 ml of hydroxylamine hydrochloride (adjusted to pH 6.0 with NaOH) were added. The auto-oxidation of hydroxylamine was observed by measuring the change in optical density at 560 nm for 2 min at 30/60 s intervals.

2.6.2.4 Estimation of catalase

Catalase activity was assayed by the method of Claiborne (1985). Briefly, the assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml post mitochondrial supernatant (10%) in a final volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of K min−1.

2.6.3 Biogenic amine (Serotonin) Estimation

Measurement of biogenic amine content in CNS tissues Biogenic amines (serotonin) was estimated by HPLC with electrochemical detector. Waters standard system consisting of a high pressure isocratic pump, a 20 µL sample injector valve, C18 reverse phase column and electrochemical detector was used. Data was recorded and analyzed with the help of empower software. Mobile phase consisting of sodium citrate buffer (pH 4.5) - acetonitrile (87:13, v/v). Sodium citrate buffer consist of 10mM citric acid, 25mM NaH2HP04, 25mM EDTA, and 2mM of 1-heptane sulphonic acid (Patel et al., 2005). Electrochemical conditions for the experiment were + 0.75 V, sensitivity ranges from 5-50 nA. Separation was carried out at a flow rate of 0.8 ml/min. Samples (20 µL) were injected. On the day of experiment frozen brain samples were thawed and they were homogenized in homogenizing solution (Mobile Phase) containing 0.2 M perchloric acid. After that samples were centrifuged at 12000 for 5 min. The supernatant was further filtered through 0.22 µm nylon filters before injecting in the HPLC injection pump. Data was recorded and analyzed with the help of empower software.

2.7 Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism version (GraphPad Software Inc., San Diego, CA, USA). Comparisons among different experimental groups were carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Qualitative phytochemical analysis

Preliminary phytochemical screening showed that there was difference in the phytochemical constituents of the aqueous and n-hexane extracts of *Fragaria* ananassa. The aqueous extract revealed the presence of alkaloids, flavonoids, phenols, tannins, glycosides, steroids and terpenoids, flavonoids, phenols and tannins were predominately detected. The other tested phytochemical classes were, however, absent in the n-hexane extract where steroids and terpenoids were the major classes present. The results suggest that the extraction solvent affected the phytochemical composition of *F. ananassa* extracts.

Table 1: Phytochemical of plant extracts

Phytochemical Class	Chemical Test Assay	n-Hexane Extract	Aqueous Extract
Alkaloids	Mayer's / Wagner's	—	+
Flavonoids	Shinoda Test	—	+++

Phenols	Ferric Chloride	—	+++
Tannins	Gelatin Test	—	+++
Saponins	Froth Test	—	++
Glycosides	Keller-Kiliani	—	+
Steroids	Salkowski Test	+++	+
Terpenoids	Salkowski Test	++	+

3.2 Effect on elevated plus maze behaviour

Treatment with *F. ananassa* extracts resulted in a gradual decrease in closed arm entries during the elevated plus maze. The aqueous and n-hexane extracts of the plants had significant differences from the normal group at specific observation points, with significant differences emerging from the diazepam treated group at later points of treatment. Overall, there was a trend toward decreased closed-arm exploration following treatment.

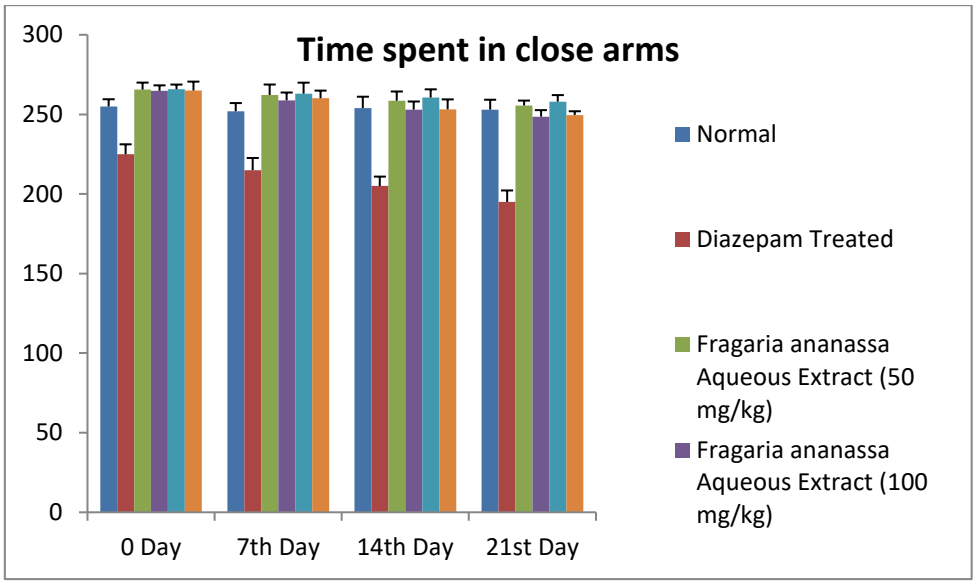

Fig. 1: Effect of plant extracts on time spent in close arms

The treatment groups with extract had a progressive increase in time spent in the open arms during the treatment period. In both extracts, the longer the animals were treated the more time they spent in the open arms, and the higher the dose, the more the animals spent in open arms during the later observation periods. There were significant differences between the extract-treated groups and diazepam-treated group at the stated observation points.

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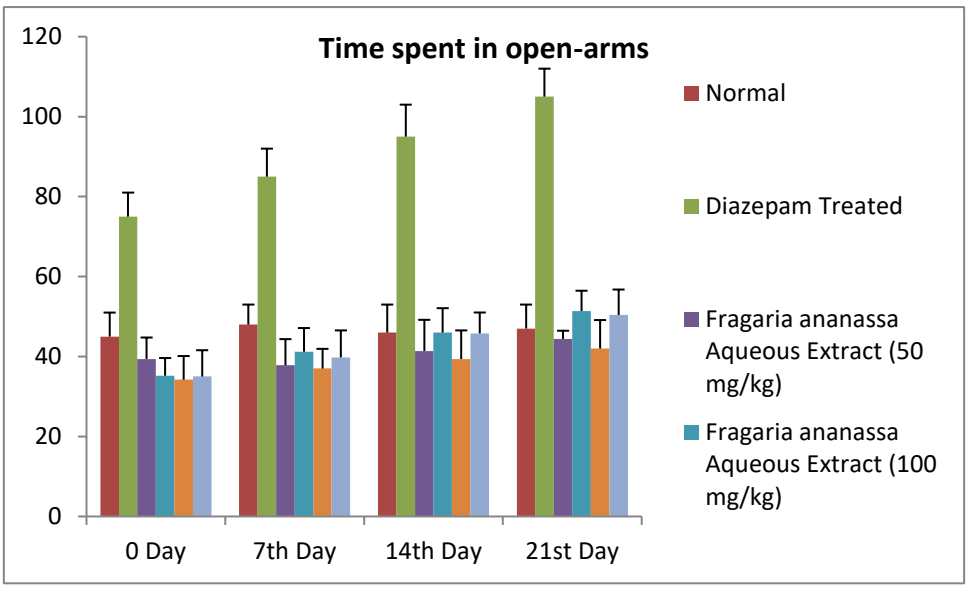

Fig. 2: Effect of plant extracts on time spent in open arms

The same trend was observed in case of the number of open arm entries that increased gradually after having been treated with both aqueous and n hexane extracts. This rise was more significant at the higher doses and at later treatment times. There were significant differences between several extract treated groups and normal group and/or diazepam treated group.

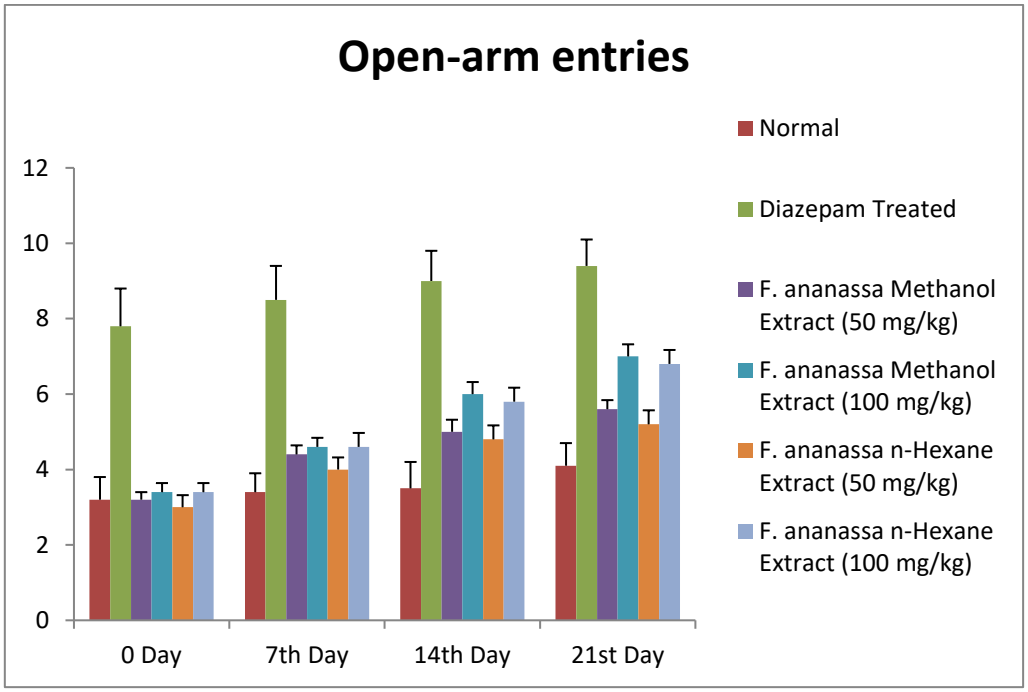

Fig. 3: Effect of plant extracts on open arm entries

The normal group's time in the closed arms was fairly maintained while the diazepam group's time in the closed arms was gradually decreased with each treatment session. The closed arm occupancy, which decreased over time in the extract-treated groups, tended to be higher than in the diazepam treated group, however. Significant differences from the diazepam-treated group were observed in the extract-treated groups throughout the experimental period.

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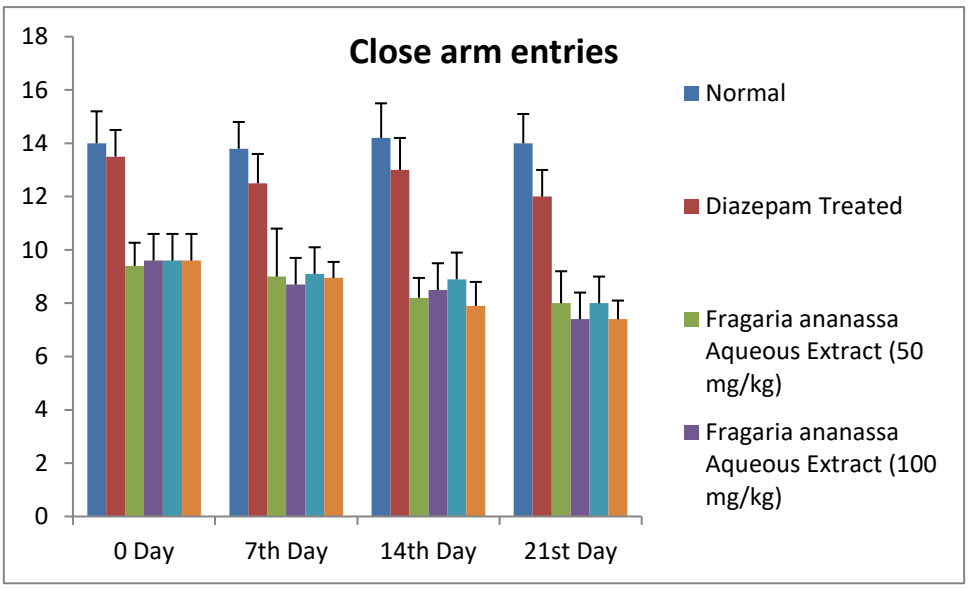

Fig. 4: Effect of plant extracts on close arm entries

3.3 Effect on light and dark box behaviour

With the light and dark model, a significant increase in the number of entries in the light compartment was obtained in the diazepam group relative to the normal group. The number of light-compartment entries for both extracts (aqueous and n-hexane) increased gradually throughout the treatment. The effect was greater for the high doses and significant differences from normal and diazepam-treated groups were seen at various observation times.

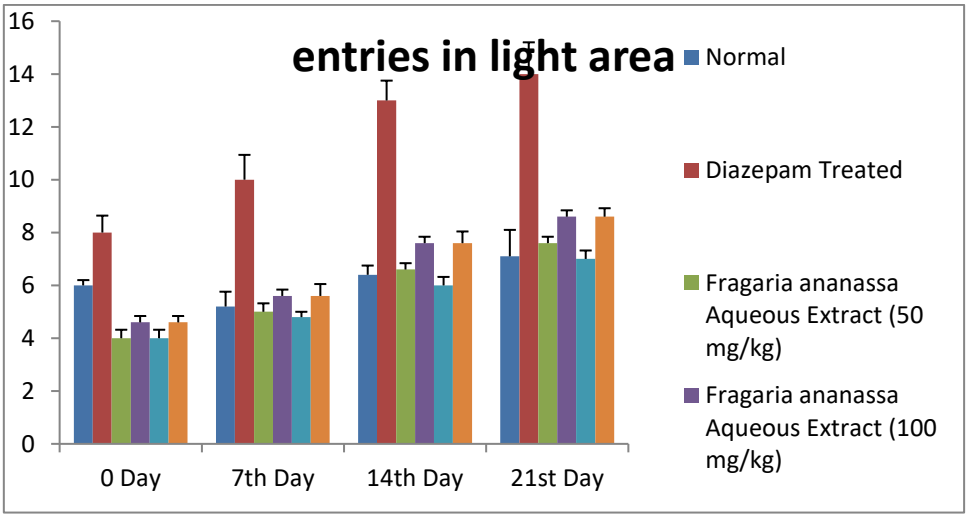

Fig. 5: Effect of plant extracts on entries in light area

Diazepam significantly increased the time spent in the light compartment as compared with the normal group. The light-compartment occupancy increased gradually throughout the treatment period in both aqueous and n-hexane extracts. In general, the higher doses of both extracts had a stronger impact at later observation times, with significant difference being seen from the reference groups at specific times.

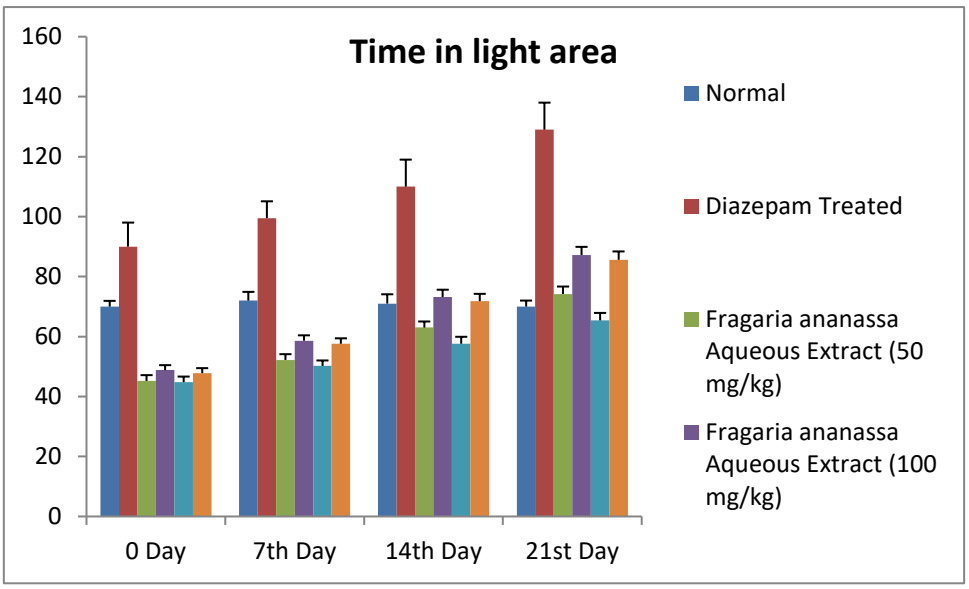

Fig. 6: Effect of plant extracts on time spent in light area

3.4 Effect on open field behaviour

The locomotor activity of the experimental groups was measured by the number of crossings over a period of 21 days, and significant differences were found between the groups. The diazepam-treated positive control group showed a significant, time-dependent increase in the number of crossings from day 7 to 21, while the normal control group did not. The aqueous and n-hexane extracts of the *F. ananassa* also showed dose-dependent increases in locomotor activity at all the time points. Of particular interest, however, was the fact that the higher dose of both extracts (aqueous and n-hexane) yielded the greatest increases at the 21st day endpoint. All of the groups treated with plant extracts demonstrated greater exploratory behavior compared to the stable baseline of the normal group, with the magnitude reaching the same or greater levels than the positive control (diazepam).

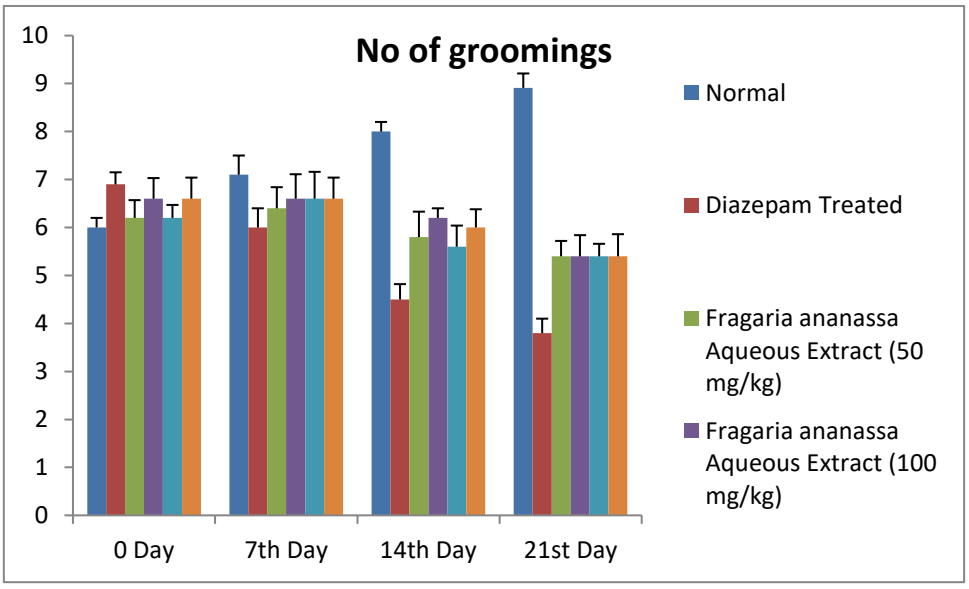

Fig. 7: Effect of plant extracts on number of grooming

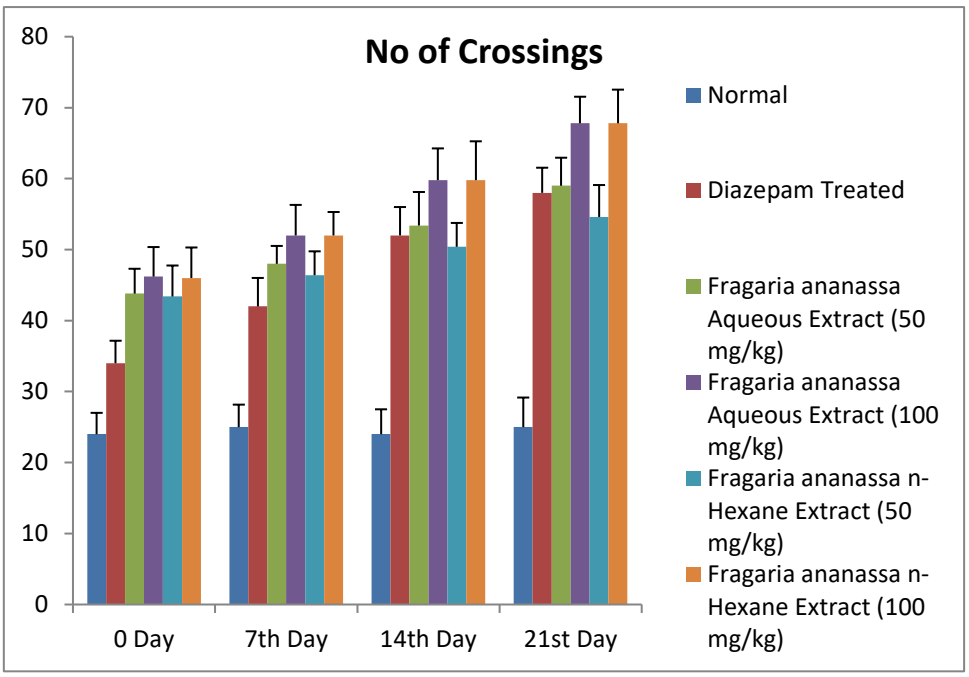

Fig. 8: Effect of plant extracts on no of crossings

3.5 Effect on oxidative stress markers

Progressive changes were observed in the open field time parameter in extract treated groups during the treatment period. Significant differences from the normal and diazepam-treated groups were mainly observed during the later treatment periods for both aqueous and n-hexane extracts.

The biochemical parameters of oxidative stress were significantly changed after treatment with extracts of *F. ananassa* and Diazepam. Diazepam treatment was found to significantly decrease MDA levels as compared to the normal group. Both aqueous and n-hexane extracts also resulted in appreciable decrease of the MDA when compared with the normal group, n-hexane extract being significantly different from the diazepam group in both doses. The same situation was also found with the aqueous extract at the lower dose when compared to the diazepam treated group.

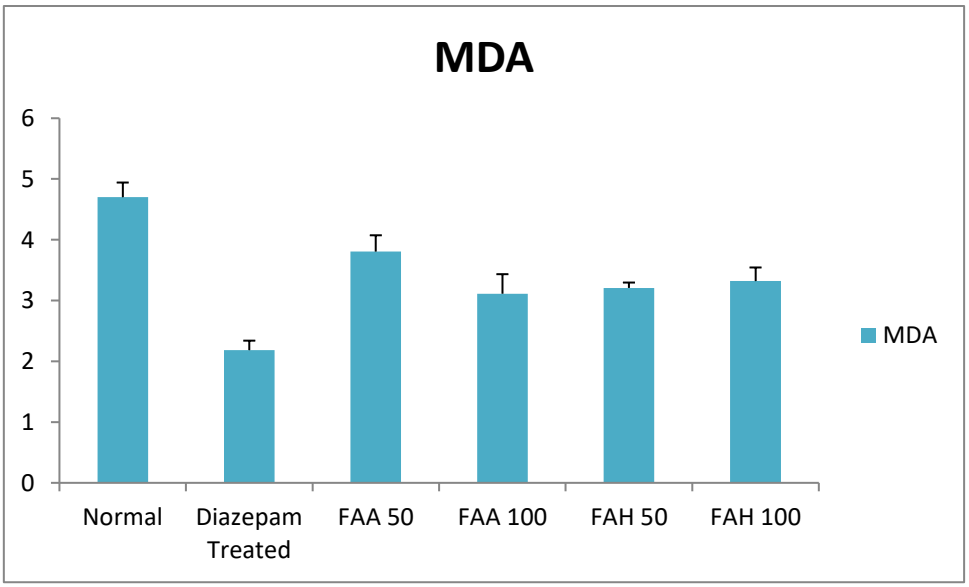

Fig. 9: Effect of plant extracts on MDA levels

The diazepam treated group showed significant increase in GSH levels than the normal group. The aqueous extract at the lower dose was significantly different than diazepam treated group while the higher dose caused a significant increase than the normal group. The n-hexane extract at both doses showed significant increase in GSH than normal group.

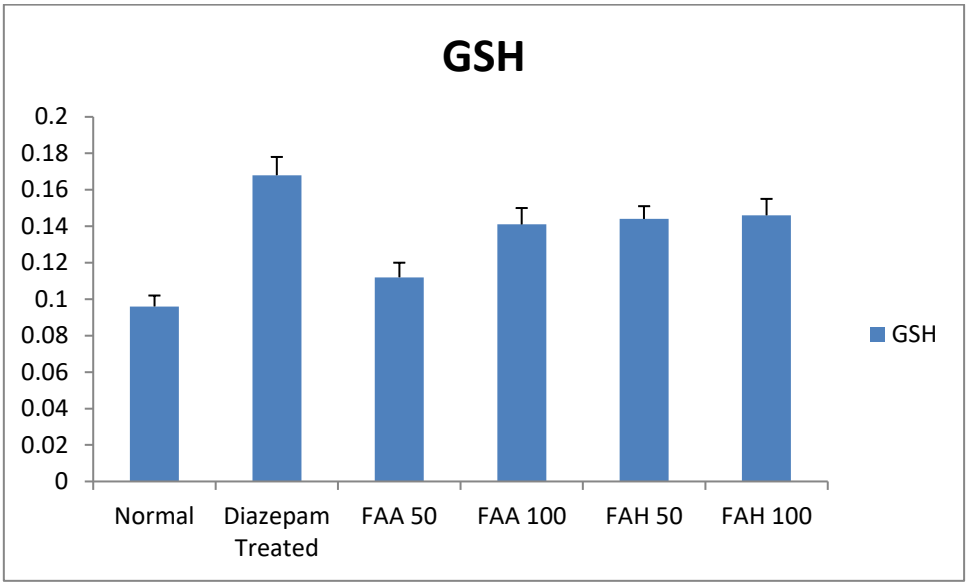

Fig. 10: Effect of plant extracts on GSH levels

The aqueous extract significantly increased the catalase activity. The aqueous extract significantly increased the values when compared with the normal and diazepam treated groups, the higher dose having more pronounced effect. The lower dose of the n-heane extract also statistically increased catalase activity, compared to both control groups.

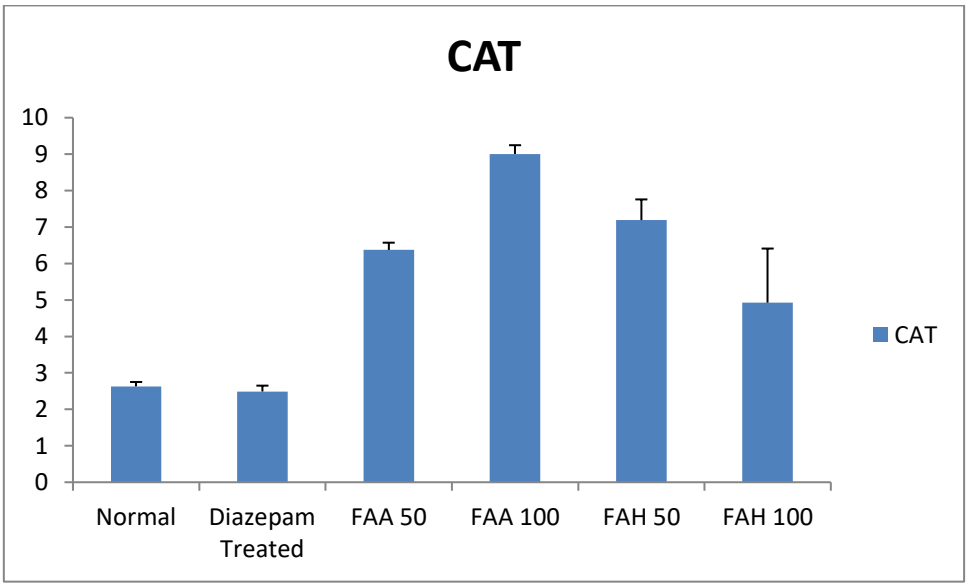

Fig. 11: Effect of plant extracts on CAT levels

Diazepam treatment was highly effective in enhancing SOD activity when compared to normal group. In both cases (aqueous and n-hexane extract), all tested doses of the extracts showed significant increase in SOD activity when compared with the normal group. The lower dose of aqueous extract also showed significant differences when compared to diazepam treated group.

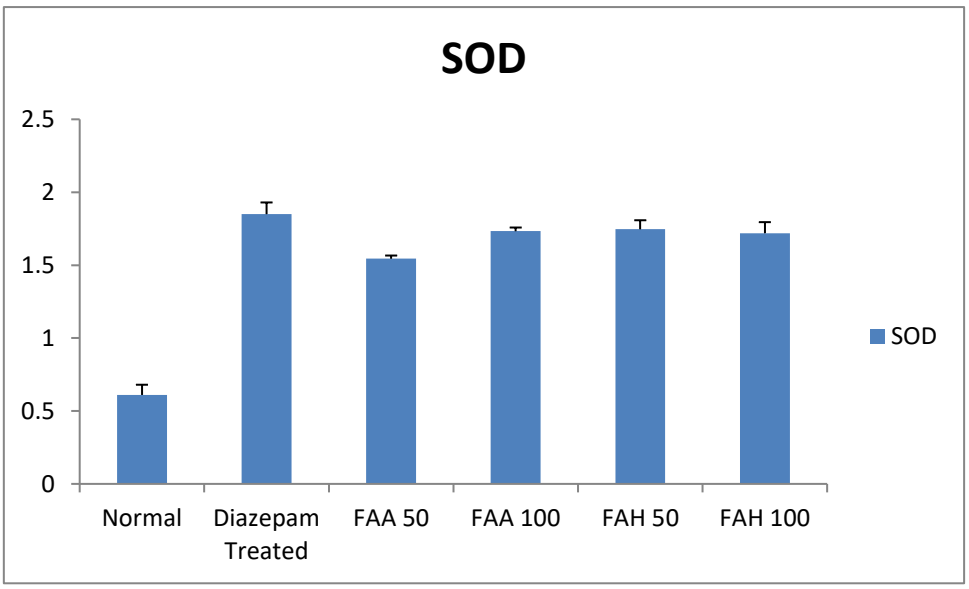

Fig. 12: Effect of plant extracts on SOD levels

3.6 Effect on brain serotonin levels

Brain 5-HT levels were significantly reduced following Diazepam treatment in comparison to the normal group. However, treatment with both aqueous and n-hexane extracts led to the significant increase in brain 5-HT levels. The aqueous extract at the lower dose showed significant difference from both the normal and diazepam treated groups. This higher dose also was significantly different from both reference groups. The n-hexane extract was also found to significantly increase 5-HT levels, both after the first and second dose, in comparison to the diazepam treated group and the normal group.

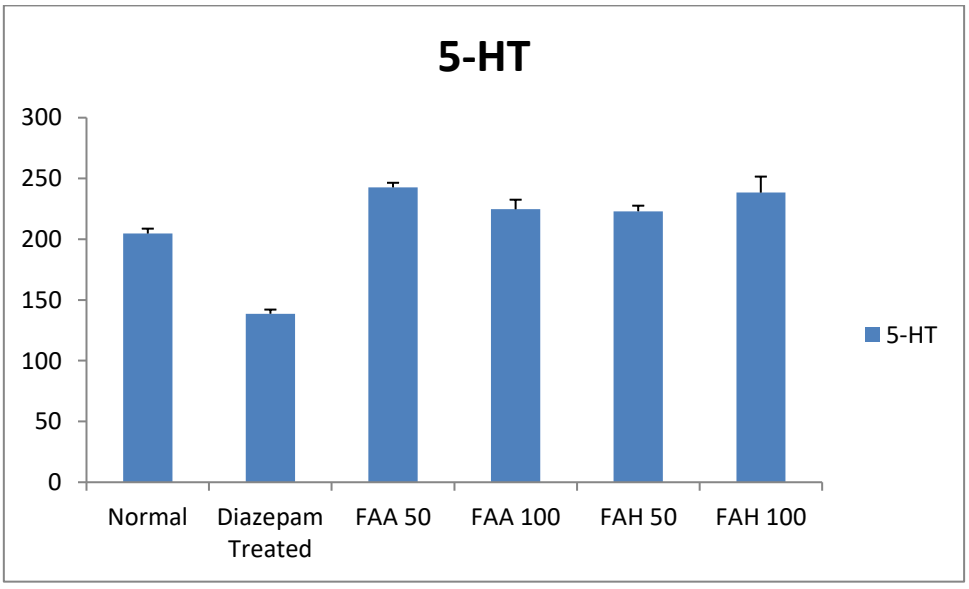

Fig. 13: Effect of plant extracts on 5-HT levels

Behavioural and biochemical changes were found in all the experimental models when treated with the aqueous and n-hexane extracts of *F. ananassa* . The extracts improved exploratory activity in the elevated plus maze and light and dark models, antioxidant related parameters, lowered MDA levels and raised 5-HT levels in the brain. Most of the behavioural effects increased with the progression of treatment, especially at the higher dose levels.

4. DISCUSSION:

In the present study, the chronic neuropharmacological potential, monoaminergic modulatory effects and central antioxidant mechanisms of aqueous and n-hexane fruit extracts of *Fragaria x ananassa* produced a significant reduction in the anxiety of these animal models, when tested in validated anxiety models. The key findings reveal progressive anxiolytic effects of both polar and non-polar extracts which were also time-dependent, with significant increases in serotonin (5-HT) levels in the brain, while also showing marked reductions in oxidative stress in the brain. The solvent used to extract plant materials can greatly affect the therapeutic efficacy of the extract by virtue of its polarity that determines the solubilization and recovery of the bioactive phytochemical classes. In this study, a clear chemical difference between the polar fraction and non-polar fraction was detected by qualitative screening. The aqueous extract showed rich concentration of the secondary metabolites, which are hydrophilic in nature such as flavonoids, phenols, tannins and saponins. Flavonoids and polyphenols are naturally occurring phytochemicals known to have neuroprotective, free radical scavenging and central nervous system (CNS) modulatory properties. On the other hand, the n-hexane extract only extracted non-polar lipophilic compounds such as steroids and terpenoids. Terpenoids and phytosterols have also been suggested to cross the blood–brain barrier (BBB) and exert effects on neurobehavioral responses and/or lipid dynamics in membranes. The Elevated Plus Maze (EPM), Light-Dark Box and Open Field Test (OFT) are exploratory rodent models that use the innate conflict between exploration of novel spaces and the aversion to open elevated or illuminated spaces of a rodent. This hesitation is decreased by standard anxiolytics, such as benzodiazepines (e.g., Diazepam) and leads to more open arm exploration and more time in the light compartment. Both aqueous and n-hexane extracts (50 mg/kg and 100mg/kg), resulted in time dependent anxiolytic-like behaviour effects during the 21 day intervention period. The EPM allowed for observation of the gradual, sustained increase in open arm duration, as well as open arm entry frequency, in extract treated animals, with a corresponding decrease in closed arm entry and occupancy. In the same way, in the Light-Dark test, both extracts increased the number of entries and the total duration of time spent in the light side in a progressive way. Importantly, the anxiolytic effect of Diazepam, which was maximum early and progressively reduced over time, was opposite to the behavioral effects of F. x *ananassa* extracts, which continued to improve over time up to Day 21. In the Open Field Test, extract administration resulted in a behavioural profile different from the profile of conventional sedatives. Typically, classic central depressants cause a reduction in total line crossings, either because of motor sedation or ataxia, over the long-term treatment. Both FAA and FAH, however, caused a gradual, time-dependent rise in line crossings without causing sedative impairment. At the same time, there was a gradual reduction in the duration of center area among extract groups. This increase in general locomotor crossings and a decrease in center occupancy indicates that they have more exploratory motivation and more habituated navigation strategies than does a state of sedation or motor incoordination. One of the most interesting findings of this study is the difference in brain 5-HT modulation between the extracts of F. x *ananassa* and Diazepam. Diazepam treatment significantly reduced brain 5-HT levels compared to controls which is consistent with feedback inhibition by GABA A in the raphe nuclei on monoaminergic projections. On the other hand, both FAA and FAH markedly raised the concentration of serotonin in the brain at all the dose levels tested. Serotonin is a master neurotransmitter that controls emotional processing, mood and stress adaptation. The ability of FAA (flavonoids and polyphenols rich) and FAH (steroids and terpenoids rich) to increase central 5-HT levels suggests that their anxiolytic activity is possibly through monoaminergic pathways, either by inhibiting monoamine oxidase-A (MAO-A) or by enhancing 5-HT synthesis, or by modulation of pre/postsynaptic 5-HT 1A receptors. This dual action of reducing anxiety and increasing brain serotonin is a pharmacological benefit over traditional sedatives that typically reduce monoaminergic tone. Central oxidative stress is tightly coupled to chronic psychological and anxiety stress, with an overproduction of reactive oxygen species (ROS) causing neuronal damage and membrane lipid peroxidation. In this study, chronic treatment with the extracts of F. x *ananassa* significantly protected brain tissue against oxidative damage: Lipid Peroxidation (MDA): It was observed that both FAA and FAH significantly reduced the level of malondialdehyde (MDA) in the brains, indicating protection from ROS-induced lipid degradation. In both treatment groups, glutathione and Superoxide Dismutase (GSH & SOD), non-enzymatic and enzymatic antioxidants, were significantly raised indicating the radical neutralising ability of neural tissue. The antioxidant markers also showed a significant upregulation of Catalase activity with a dose-dependent manner for FAA. The strong effect is directly related to the high phenolic and flavonoid content of FAA that can activate the Nrf2/ARE signalling pathway leading to the upregulation of endogenous Phase II antioxidant enzymes.

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