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Effect of *Aloe vera* on brain indoleamine 2, 3 dioxygenase activity and tissue antioxidant status in rats subjected to swim stress

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Abstract: *Aloe vera* (AV), is a succulent plant with numerous health benefits, including antioxidant, immune-boosting, and antidepressant properties. Increased brain indoleamine 2, 3-dioxygenase (IDO) activity due to proinflammatory cytokines contributes to serotonin reduction and depression. This study investigates how AV affects brain IDO activity and liver antioxidant status in rats subjected to a forced swim test (FST). Albino Wistar rats were assigned to control and AV-treated groups (n=12/group). The test group received an aqueous extract of AV orally at 0.2g/ml/kg, while the control group received tap water for fourteen days. Behavioral analysis showed AV's anxiolytic properties in mice subjected to an elevated plus maze (EPM) test, with a significant reduction in total locomotor activity and exploratory behavior in an open field test. An antidepressant effect was indicated by decreased ($p<0.05$) immobility time in FST and decreased brain IDO activity in AV-treated rats. Moreover, the significant antioxidant activity of AV was reflected in elevated catalase and reduced glutathione (GSH) levels, along with considerable depletion in malondialdehyde (MDA) levels when compared to unstressed controls. Together, these findings suggest AV possesses potent antioxidant and anxiolytic properties, mitigating stress-induced depressive states by decreasing brain IDO activity, thereby increasing tryptophan availability for central serotonin synthesis.

Keywords: *Aloe vera*, antioxidant, anxiolytic, indoleamine 2, 3 dioxygenase, depression.

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INTRODUCTION

Aloe vera (AV) from the family *Asphodelaceae* (Liliaceae) is a succulent plant with health benefits (Pathak and Sharma, 2017). Its three-layered leaf is made up of 99% water in the inner gel layer and amino acids, lipids and vitamins in the outer two layers. Its exterior thick layer, which serves as a shielding layer and is made up of proteins and carbohydrates, is composed of latex, a bitter pale secretion that contains anthraquinones and glycosides (Surjushe *et al.,* 2008). AV leaf gel contains amino acids, minerals, and polysaccharides (Mazzulla *et al.,* 2012) which are reported to exhibit many biological functions such as anti-diabetic, detoxifying, immunity boosting properties and anti-damaging effects for hepatocytes (Nandal and Bhardwaj, 2012).

Tryptophan (TRP) is an essential amino acid crucial for protein synthesis. In adults, the daily consumption of TRP ranges from 4 to 5 grams. (Platten *et al*., 2019) It plays a role in regulating appetite, mood, immunity and behavior (Guillemin *et al.,* 2007). TRP is the progenitor of two important metabolic pathways, kynurenine (KYN) synthesis and serotonin synthesis. Approximately 95% of ingested tryptophan (TRP) is metabolized to kynurenine (KYN), facilitated by two enzymes that act as inherent rate-limiting steps. These enzymes include hepatic tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO), which is present in tissues beyond the liver (Badawy, 2017). The unbound fraction of plasma tryptophan (TRP) serves as a critical peripheral factor for TRP entry into the brain, where it is utilized in the synthesis of cerebral 5-hydroxytryptamine (5-HT), commonly known as serotonin. Previous research has demonstrated that all antidepressants elevate free serum TRP levels, resulting in an increase in brain TRP concentration (Badawy, 2013) and an inverse relationship of peripheral TDO with brain TRP has previously been reported (Bano *et al.,* 2010). There is a role of TDO in anxiety-related behavior (Kanai *et al*., 2009). Increased degradation of TRP may lead to depleted levels of serotonin which becomes the cause of depressive symptoms (Russo *et al.,* 2003) and ultimately may lead to oxidative stress due to the activation of TRP metabolizing major pathway. Evidence suggests that both TRP and the resulting serotonin depletion, as well as the induction of indoleamine 2,3-dioxygenase (IDO), contribute to the pathophysiology of depressive illness (Maes *et al*., 2011). The upregulation of indoleamine 2,3-dioxygenase (IDO) due to proinflammatory cytokines, lipopolysaccharides, and oxidative stress may contribute to mood disorders associated with inflammation. Antioxidants, on the other hand, exhibit hepatoprotective effects by counteracting oxidative damage caused by reactive oxygen species (ROS) (Kumar *et al.,* 2017). These free radicals are superoxide, hydroxyl, alkoxyl, and hydroperoxyl that elevate the oxidative stresses (Lu *et al.,* 2010; Han *et al.,* 2012) and cause an abnormality in naturally producing

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anti-oxidants thus triggering apoptosis and oxidative degradation of lipids as well (Gill and Tuteja, 2010). As AV has potent bioactive substances with anti-inflammatory and antidepressant properties, therefore, we aim to examine the impact of AV pretreatment on the brain kynurenine (KYN) pathway and behavioral and liver antioxidant status in swim-stressed rats

MATERIALS AND METHODS

Animals

The study was designed at the Department of Biochemistry, University of Karachi, Pakistan. Twenty-four locally bred male 150–170g Albino Wistar rats were acquired from the International Centre for Chemical and Biological Sciences (ICCBS) University of Karachi. All rats were kept at 22±2°C with easy availability of tap water and standard pellet diet and were kept at a 12-hour day-night cycle at 50 to 60 percent moisture. Procedures regarding animal treatment were performed according to the guidelines for the usage and maintenance of experimental animals issued by the National Research Council in 1996. The University of Karachi's animal ethics committee, BASR/No. /04011/Sc. granted ethical approval for the present study.

Plant material and preparation of extract

Recently harvested leaves of AV with a resilient and vibrant growth, were collected and authenticated by the Herbarium, University of Karachi. AV crude gel was extracted by removing the hard outer covering and cutting out the gel with the sterile instrument into tiny slices. A particular amount of gel was homogenized with distilled water (1:5 w/v) to obtain a crude gel extract (Paul *et al.*, 2017). Homogenate was then stored in Eppendorf's tubes at -20ºC and tubes to be used were defrosted before administration.

AV treatment

After a week of acclimatization rats were randomly assigned control and AV-treated (n=12 in each group. Tap water was given to the control groups, while other groups received a dosage of 0.2g/ml/kg body weight. Aqueous AV extract orally for 14 days, body weight and food intake were monitored throughout treatment.

Behavioural assessment

On the 14th day of treatment, rats were subjected to behavioural analysis for anxiolytic activity by observing time spent in the open arm in elevated plus Maze (EPM) test (Pellow *et al*., 1985) and activity in Open field test (OFT) (Walsh and Cummins, 1976) were recorded to assess locomotor activity and gross exploratory behavior. Animals for behavioural analysis were separate from those used for biochemical analysis.

Forced swim test (FST)

The FST pre-test was performed between 9:00 am -1 pm on day 13th of treatment with 15 min for every rat, followed by a test after 24 hours on day 14th. Test rats received their last dose 30 min before the test session. FST was performed by placing the rat into a cylindrical water tank (height 45 cm, diameter 30 cm), made of Plexiglass (transparent), containing tap water at 25ºC, for forced swimming (Porsolt *et al*., 1977). The tank was large enough to stop the rats from escaping from the tank top as well as touching the tank base with their tails. The total recording time was 5 min, as soon as the rat was placed in the water. All FST test sessions of both control and treated rats were videotaped, for later analysis. The duration of passive behavior (immobility) and active behaviors (climbing and swimming) were recorded. When rats stopped struggling and stayed floating in a motionless manner in the water and showed complete absence of the entire body's motion then this condition was marked as immobility. When rats displaced their bodies around the water tank by performing large and parallel movements of the forepaws, this action was recorded as swimming. Climbing was noted when rats moved their bodies around the tank by displaying strong vertical movements of the fore-paws, directed as opposed to the tank wall.

Biochemical analysis

Sample Collection

On the 14th day of treatment AV the antidepressant effect of AV was seen in rats by monitoring time spent while swimming, immobility, and climbing in a forced swim test (FST) (Porsolt *et al*., 1977), Animals were decapitated, whole brain and liver tissue isolated were kept at -70ºC until analysis. Blood was allowed to stand for 30 minutes in centrifuge tubes. Serum was also isolated after centrifugation and stored at -20ºC until analysis.

Preparation of tissue homogenates

Liver tissue (1g) was homogenized in 0.1M phosphate buffer (pH 7) using polytron-PT-2100 to prepare 10% homogenate. The homogenate was used to measure the amounts of antioxidant indicators such as catalase, GSH, MDA and total protein (Lowry *et al.*, 1951) using a UV/VS spectrophotometer (TOMOS, USA, model UV 1600). After weighting, the frozen brain tissues of each rat were homogenized in 12% HClO4 in ice-cold deionized water for 5 sec in a homogenizer. Homogenates were then sonicated in a bench-top ultrasonic cleaner (.603) filled with ice-cold distilled water for 10 min. Centrifugation of homogenates was done for 15 min at 10,000 rpm in a refrigerated centrifuge machine at 4ºC. Supernatants were immediately used for measurement of tryptophan and kynurenine contents in samples through high-performance liquid chromatography (HPLC).

Evaluation of the liver's antioxidant levels

The quantity of Malondialdehyde (MDA) in liver tissue homogenates was quantified using the thiobarbituric acid (TBA) reaction (Mihara & Uchiyama M, 1978), Catalase

by (Sinha 1973) Reduced glutathione GSH as described in detail previously (Hamid *et al*., 2023).

Brain IDO activity

The ratio of KYN/TRP is used to determine brain indoleamine 2,3 dioxygenase (IDO) activity Concentration of KYN and TRP in brain tissue extracts was quantified through the utilization of High-Performance Liquid Chromatography (HPLC) connected to UV/FL detector by the method of Badawy and Morgan (2010) as described in detail (Bano *et al*., 2021).

Chemicals

L-Tryptophan (≥98%), L-Kynurenine (≥98%), 5-Hydroxytryptamine hydrochloride (5-HT) ≥98%, Perchloric acid ACS reagent, 70% were purchased from Sigma Aldrich. All other chemicals were of commercially pure grade.

STATISTICAL ANALYSIS

Data was analyzed by using the statistical software program IBM-SPSS Statistics 24. The values are expressed as means ± SEM. A Student's t-test was employed to compare the two groups. Multiple comparisons were conducted and analyzed using two-way ANOVA, followed by post hoc Newman-Keuls or Tukey's test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Effect of AV on Brain Tryptophan, kynurenine and IDO activity in rats

Fig. 1 (A-C) illustrates the impact of AV on the brain kynurenine pathway in rats. A two-way ANOVA was performed to evaluate the effects of stress and drug administration. Brain TRP, Brain KYN and IDO activity (KYN/TRP) were measured under unstressed and stressed conditions, with and without drug treatment. The two-way ANOVA analysis revealed a significant ($p<0.001$) impact of stress, drug treatment, and interaction between stress and drug treatment on brain TRP, KYN and IDO activity.

Effect of AV on liver total protein and antioxidants in rats

Fig. 2 (A-C) shows the effect of AV on hepatic oxidative stress in unstressed and stressed groups of rats by measuring the levels of antioxidant markers i.e. catalase, GSH, MDA, and total proteins. Stress appears to induce a significant reduction in catalase with a potent rise in MDA levels. However, a substantial rise in catalase and GSH along with a significant reduction in MDA concentration was observed upon AV treatment in both unstressed and stressed groups of rats. Statistical analysis showed a noteworthy ($p<0.01$) effect of AV on Catalase ($F=44.280$), GSH ($F=36.72$) and MDA ($F=25.34$) with a significant effect of stress on Catalase ($F=7.73$, $P<0.01$) and MDA levels.

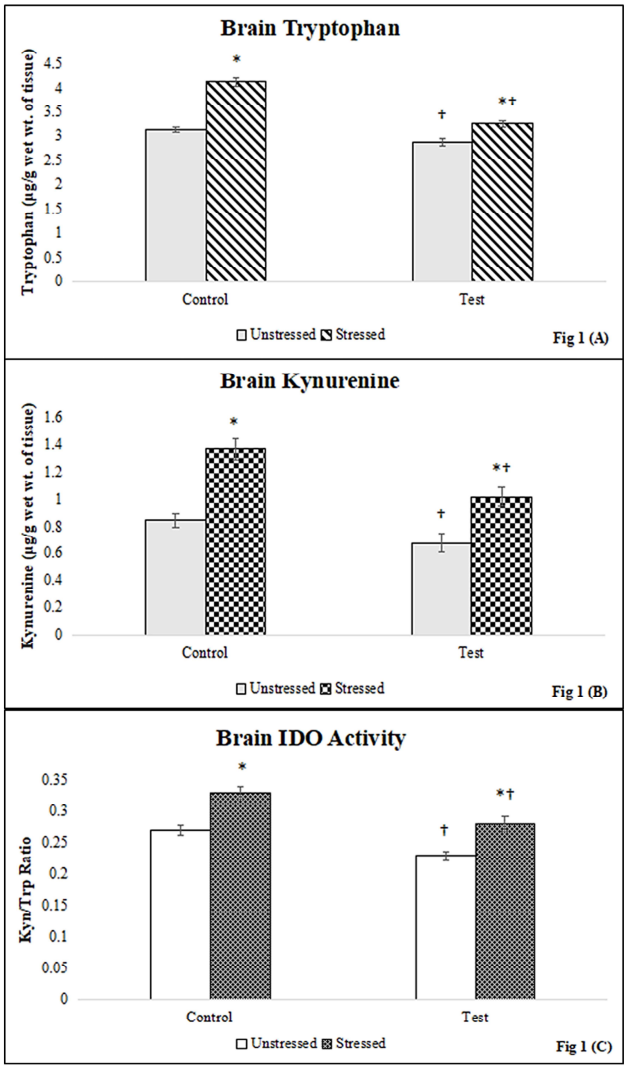


Fig. 1: (A-C) shows the effect of AV on brain tryptophan, Kynurenine, and IDO activity in Forced Swim rats. Data was analyzed using Two-way ANOVA and post hoc analysis was done using Newman-Keuls q-test. Significant differences are denoted by *p is less than <0.01 when comparing stressed groups vs respective controls and † (p is less than 0.01) when comparing AV-treated groups vs similarly treated control group.

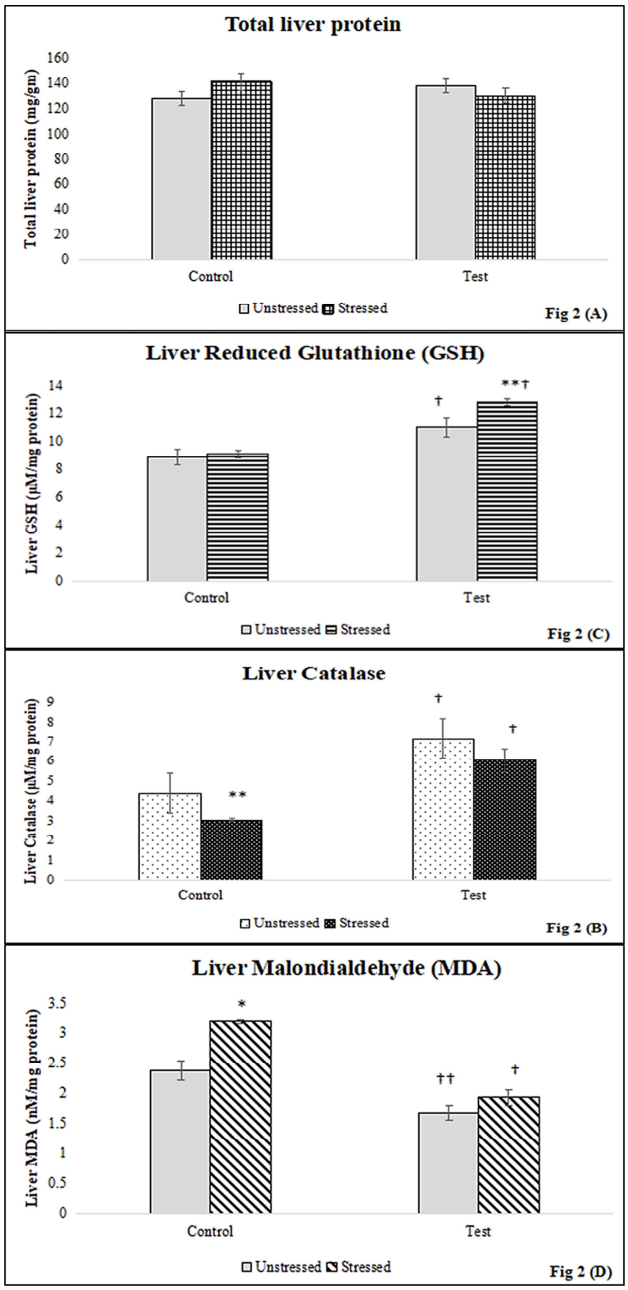
Effect of AV on rats subjected to OFT

Fig. 3 depicts the effect of AV on locomotion/ exploratory behavior in rats, evaluated by measuring the total locomotion for 30 minutes by each rat. A significant reduction in total locomotor activity/ exploratory behaviour ($p<0.001$) of rats was observed in half an hour when compared with their respective controls.

Effect of AV on rats subjected to FST

Fig. 4 shows the antidepressant effects of AV in rats, evaluated by measuring the duration of swimming,

climbing and immobility in FST. Immobility and climbing time were decreased, while swimming time was significantly increased by the AV-treated group compared with controls.



Data presented was analyzed using two-way ANOVA and post hoc analysis by Newman-Keuls q-test. Significant differences are denoted by *p<0.05 and **p<0.01 when comparing stressed groups vs respective controls, and †p<0.05 and ††p<0.01 when comparing AV-treated groups vs similarly treated controls.

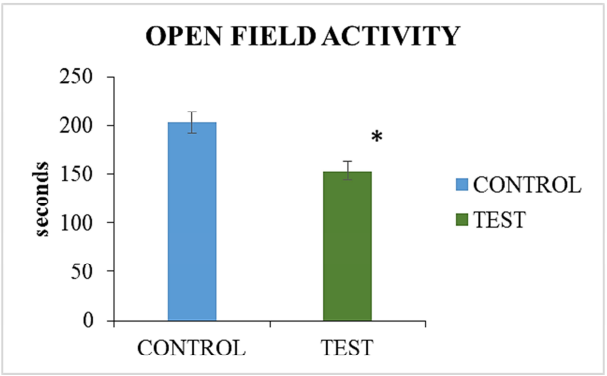
Fig. 2: (A-C) shows effects of AV on antioxidant status on rats

Effect of AV on rats subjected to EMP test

Fig. 5 shows the anxiolytic effect of AV in rats, evaluated by measuring the time spent (sec) in the open arm of EPM. It was observed that the AV-treated rats spent more time (p<0.01) in open arms than their control group.

DISCUSSION

Impact of prolonged AV administration on anxiety-like behaviors, brain IDO activity and hepatic antioxidant status in rats subjected to swim stress. Exploratory behavior was observed following AV treatment by assessing locomotor activity in OFT compared to respective controls. A significant reduction in the number of squares crossed after AV treatment suggests reduced locomotion which might be indicative of altered behavior, potentially linked to the decrease in anxiety-like behavior reflected in open-arm exploration in EPM. Our findings align with those of a previous study suggesting an anxiolytic effect of AV (Sultana & Najam, 2012). Furthermore, increased swimming by AV-treated rats in the present study could suggest a shift towards more active coping strategies in FST which is one of the crucial tests and is widely used in preclinical studies (Slattery & Cryan, 2012) to measure depression-like behaviour. Decreased immobility and climbing duration might also reflect a less passive response to stress and an overall depletion in anxiety-related behavior. Such changes in swimming, immobility and climbing behaviors point out a shift in coping strategies and potentially reduced stress-induced responses.

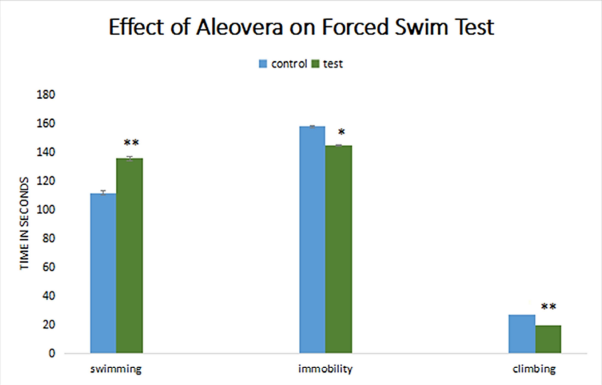


Data are expressed as mean ± SEM for each group of 6 rats. A student's t-test was performed. Significant differences are denoted by *p<0.01 when comparing the AV-treated group vs the control group.

Fig. 3: Effect of AV on exploratory behavior of rats in Open Field Test (OFT).

Present findings align with those of a previous study which shows that pure extract of AV reduces depression in pre-diabetics upon eight weeks of treatment (Foadoddini and Mofrad, 2020) with the endorsement of another study reduction in immobility time in FST at different AV doses 100-400 mg/kg/ml (Halder *et al*., 2013) in mice. Such results proposed that AV treatment has the potential to mitigate the adverse effects of swim stress.

In swim stressed group of rats, brain IDO activity was found to be notably increased which was found to be potentially coped by AV-treated rats when subjected to swim stress. These observations suggest that AV treatment has the potential to modify stress-induced disruption in tryptophan metabolism through the kynurenine pathway. We are the first to report that IDO activity is upregulated in stressed rats. It could be suggested that this enhanced IDO activity may lead to a decrease in the 5HT levels in the brain, leading to depression. It is suggested that IDO may be a therapeutic target for stress-related depressive disorders. There is substantial evidence that both dioxygenase enzymes are activated during depression. Consequently, these enzymes inhibition by drugs may be therapeutic targets for such illnesses (Qin, 2018).



Data shows mean ± SEM (n=6) rats. The significance of differences is denoted by **p<0.001 and *p<0.05 when comparing the AV-treated group vs the control group using the student's t-test.

Fig. 4: Effect of AV on the behavior of rats in Forced swim test (FST).

Data is expressed as mean ± SEM for every group of 6 rats. Statistical analysis was performed using Student's t-test. The significance of differences is marked with *p<0.01 when AV treated group was compared with the control group.

Fig. 5: Effect of AV on anxiety-like behavior of Albino Wistar rats in EPM test.

Rats subjected to FST, prompted a substantial increase in brain IDO Activity, highlighting its sensitivity to stressors. Chronic stress increases pro-inflammatory cytokines and simultaneously stimulates the HPA axis thereby adding to the stress response (Leonard, 2005). It has been shown that exposure of animals to psychological stress elevates brain KYN and TRP levels with no change in IDO activity. Further, sudden stress decreases the 5-HT/TRP ratio. (Miura, 2011).

AV treatment was found to reduce IDO activity, thereby exhibiting an antidepressant-like effect in rats. Any change in the concentration of serum TRP alters brain TRP concentration which in turn, regulates the rate of brain serotonin synthesis (Richard *et al*., 2009). These findings have also been elucidated by another study via a significant rise in brain TRP upon swim stress that led to intricate increases in 5-HT turnover in different brain areas (Ara, 2012).

A previously conducted study reported that IDO reduces the amount of tryptophan, available for the production of serotonin which is directly related to depression (Gałecki and Talarowska, 2018).

There is a strong association between IDO and oxidative stress. In many cell types, IDO is strongly induced during an inflammatory response, which includes interleukin-induced indole amine 2,3-dioxygenase (IDO) activation that consequently induces depression and lung cancer (Tang *et al*., 2023). Such dysregulation in the kynurenine pathway may induce oxidative stress as well. The comprehensive analysis of hepatic total protein, Catalase, GSH and MDA levels in response to stress indicated complex biochemical interactions. Stress seemed to influence oxidative stress markers as depicted by a significant reduction in liver catalase and a profound rise in MDA levels. AV treatment intervened in this stress-induced oxidative burden and the variation in the levels of oxidative stress markers is observed by a significant increase in liver catalase, and GSH with a pronounced reduction in MDA levels in both stressed and un-stressed groups upon AV administration thus, showing potential modulation in oxidative stress. Our findings are consistent with a previous study that suggests the anti-oxidative properties of AV gel may protect against oxidative damage (Haritha *et al*., 2014). AV extract has a beneficial protective effect against radiation-induced oxidative stress (Nada *et al*., 2013). Such interplay between oxidative stress and biochemical markers suggests the involvement of cellular responses to stress and interventions.

CONCLUSION

It is concluded that AV treatment in stressed rats exerts anxiolytic activity by attenuating stress-induced increases in brain IDO activity in addition AV ameliorates oxidative stress in hepatocytes. Taken together, it is suggested that AV has cognitive, anti-oxidative and

neuroprotective effects thus beneficial in coping with stress.

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