Asian Journal of Research in Medical and Pharmaceutical Sciences

3(2): 1-8, 2018; Article no.AJRIMPS.38132
ISSN: 2457-0745

Evaluation of Anti-stress Activity of *Orthosiphon stamineus Benth.* Lamiaceae Aqueous Leaves Extract on Wistar Albino Rats

Krupavaram Bethala[1*], Ragaventhiran Mohan[1], Shopana Paramasivam[1], Rama Supriya Veeralla[1] and Anandarajagopal Kalusalingam[1]

[1]School of Pharmacy, KPJ Healthcare University College, Kota Seriemas, Nilai 71800, Negeri Sembilan, Malaysia.

Authors' contributions

This work was carried out in collaboration between all authors. Authors KB developed the protocol, designed the experimental study. Authors RM and SP involved in the collection of literature, preparation of extract and screening of phytoconstituents present in the extract. Authors KB and RSV involved in the evaluation of anti-stress activity and biochemical analysis. Authors KB, RM and AK involved in data interpretation and performed the statistical analysis. Authors KB, RM and SP wrote the first draft of manuscript. Authors AK and RSV edited and completed the final manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRIMPS/2018/38132
Editor(s):
(1) Imran Aslan, Bingol University, Bingol, Turkey.
Reviewers:
(1) Vanessa de Andrade Royo, Universidade Estadual de Montes Claros, Brazil.
(2) H. L. Muhammad, Federal University of Technology Minna, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/23752

Original Research Article

Received 13th November 2017
Accepted 8th February 2018
Published 21st March 2018

ABSTRACT

Aim: The present study was aimed to determine the in-vivo anti-stress activity of *Orthosiphon stamineus* aqueous leaves extracts using Wistar albino rats.
Methodology: In anoxia stress tolerance test and noise induced stress test Wistar albino rats of either sex was randomly divided into five groups of four animals each. Aqueous leaves extract of *Orthosiphon stamineus* (200 mg/kg and 400 mg/kg p.o) was administered for 21 days for anoxia stress tolerance method, whereas 15 days for noise induced stress method and last day, blood was collected by retro-orbital route to estimate blood cell count and biochemical parameters

**Corresponding author: E-mail: kruparajs@gmail.com;*

(glucose, cholesterol, triglycerides, BUN & ACTH) to assess the anti-stress activity in Wistar albino rats. *Withania somnifera* (WS) (100 mg/kg p.o) was selected as reference standard.
Results: Results are expressed as mean ± SEM and data were analysed by Student's t-test. From the results, it was found that aqueous extract of *Orthosiphon stamineus* has been significantly increased in blood cell count and other biochemical parameters in both anoxia stress tolerance and noise induced stress test which is almost comparable to the standard drug. Furthermore, anoxia stress tolerance is more significant in the majority of the parameters when compared to noise induced stress.
Conclusion: The results indicate that aqueous leaves extract of *Orthosiphon stamineus* possesses significant anti-stress activity.

Keywords: Orthosiphon stamineus; anoxia stress tolerance; noise induced stress; Withania somnifera; student's t-test.

ABBREVIATIONS

PO : Peri oral route
RBC : Red Blood Cell;
WBC : White Blood Cell;
DLC : Differential Leukocyte Count;
BUN : Blood urea nitrogen;
ACTH : Adrenocorticotrophic hormone.

1. INTRODUCTION

Stress is a nonspecific response of the body known to change the physiological homeostasis of the human being resulting in different neuronal, endocrine, and visceral malfunctions. The capacity to develop and maintain resistance against a variety of stressors identified in human life is crucial for survival [1].

When stress becomes life-threatening, it is injurious to the body, hence, need to be treated. Stress is complex in the pathogenesis of a variety of diseases that contains psychiatric disorders such as depression, immunosuppression, anxiety, endocrine disorders including cognitive dysfunction, male impotence, peptic ulcer, diabetes mellitus, ulcerative colitis and hypertension [2].

The term adaptogens mean an agent that recovers adaptation ability of the organism during stress and anti-stress means an agent which abolishes or stops ill effects of stress and recovers adaptation. The potential efficacy of safer and economical herbal medicines as anti-stress agents has been stated in literature review. Numerous plants are known to show reliable medicinal properties for the treatment of stress and need to be discovered to identify their potential application in inhibition and therapy of human ailments [3].

Orthosiphon stamineus or Misai Kucing is a folklore herb that is widely grown in humid areas [4]. It was classically consumed as a herbal tea [5] and it is believed that *Orthosiphon stamineus* leaves have diuretic properties and has been used to remove stones from the kidneys. It is also widely applied to cure rheumatism, fever, hepatitis, gallstones, eruptive, hypertension, diabetes, epilepsy as well as encouraging health and well-being [6].

The aim of this study was to determine the *in vivo* anti-stress activity of *Orthosiphon stamineus* leaves extract on Wistar albino rats because there is not much evidence about the scientific data for its anti-stress activity even though this plant has been used in folklore medicine for relieving some stress-related disorders. Hence, this study is to discover the plant extract with significant anti-stress activity.

It is hypothesized that any herbal extracts rich in adaptogenic phytoconstituents will possess significant anti-stress activity as it will be a new drug for effectively combating the problem of stress in daily human life. Thus, this plant was evaluated for anti-stress activity in stress induced Wistar albino rats for the first time.

2. MATERIALS AND METHODS

2.1 Plant Material

Orthosiphon stamineus leaves, of white flower variety, were selected. The *O. stamineus* leaves were purchased from the local supplier from Seremban, Malaysia in the month of September 2016. The leaves were authenticated by Pharmacognosist, KPJ Healthcare University College, Nilai, Negeri Sembilan, Malaysia (Reference No: KPJUC/CRI/PA/2016(24)).

2.2 Preparation of Plant Extract

The plant leaves were dried in shade at room temperature. The dried leaves were subjected to size reduction by using blender, to make it into coarse powder. Then, the powdered leaves (500 g) were extracted with aqueous solvent by using cold maceration technique for 10 days. At the end of extraction, the extract was collected by filtration using Whatman filter paper [7]. The filtered extract was evaporated and concentrated using rotary vacuum evaporator under reduced pressure to get semisolid mass and kept in desiccator until further use [8].

2.3 Preliminary Phytochemical Screening

The qualitative chemical tests were performed for identifying the presence of phytoconstituents such as alkaloids, carbohydrates, glycosides, proteins, amino acids, sterols, fixed oils, fats, phenolic compounds, tannins, triterpenoids, saponins, gums, mucilage and flavonoids in crude aqueous extract of *O. stamineus* leaves using standard methods [9].

2.4 Pharmacological Screening

2.4.1 Animals

Healthy adult Wistar albino rats weighing 200-250 g were obtained from the animal house of the KPJ Healthcare University College, Nilai, Malaysia. The animals of either sex were well ventilated and maintained under standard husbandry conditions at an ambient temperature (25±2°C) had 12 hour day and night schedules with a spacious, hygienic cage during the course of the experimental period. The animals were fed with standard pellets and water *ad libitum* during the study period [10]. The experimental protocol has been approved by KPJUC Animal & Ethical committee (Reference No. KPJUC/CRI/BPS/EC/2016/16).

2.4.2 Anoxia stress tolerance test

2.4.2.1 Principle and procedure

All the body functions, including cellular respiration, depends on the oxygen supply. Any lack of vital element will play havoc on the all body mechanisms and increases in adaptation during stress by any drug could be considered as its major anti-stress effect. During stress, adaptogens are capable of increasing succinate dehydrogenase in the brain. The enzyme is responsible for utilization and conversation of energy in the cellular system of the organism, which helps in adaptive process during stress. Each animal was kept in the hermetic and the time was noted. As the animal showed the first convulsion, it was immediately removed from the vessel and resuscitated if needed. The time duration from the entry of the animal to the hermetic vessel and appearance of the convulsions was noted and a delay of even one minute in the removal of the animals may lead to death of the animal. After one week of the drug (extract & standard) treatment, animals were once again exposed to the anoxia stress. Depending on their capacity of tolerance, the animals were observed for the 2nd and 3rd week with the same treatment, change in the time duration of anoxia tolerance was noted. On the 4th week, blood was collected from retro-orbital route to estimate biochemical parameters like blood glucose, triglycerides, cholesterol, BUN, ACTH and blood cell count (RBC, WBC, and DLC) [11].

2.4.2.2 Experimental design

The Wistar albino rats (200-250 g) of either sex were randomly divided into five groups of four each. The different groups have been assigned as below:

Group I : Normal control (Normal saline-0.1mL)
Group II : Stress control (Negative Control)
Group III : Standard group (*Withania somnifera*/Ashwagandha) (100 mg/kg)
Group IV : *Orthosiphon stamineus* extract (Aqueous) (200mg/kg)
Group V : *Orthosiphon stamineus* extract (Aqueous) (400mg/kg)

2.4.3 Noise induced stress

2.4.3.1 Principle and procedure

Noise stress in laboratory rats can be produced by loudspeakers (15 W), driven by a white noise generator (0-26 kHz), installed 30 cm above the cage. Thus, a noise level can be set at 100 dB or above uniformly throughout the cage and can be monitored by a sound level meter. Each animal to be treated is exposed to noise stress for 4 hour day for 15 days. Control group rats are also kept in the above described cage during the corresponding period of time, without noise stimulation to avoid the influence of handling

stress on an evaluation of effects due to noise exposure. The effect of noise stress exposure can be determined by estimating various biochemical parameters. On the 16th day, blood will be collected from retro-orbital route to estimate blood glucose, triglycerides, cholesterol, BUN, ACTH and blood cell count (RBC, WBC, and DLC) [12].

2.4.3.2 Experimental design

The Wistar albino rats (200-250 g) of either sex were randomly divided into five groups of four each. The different groups have been assigned as below:

Group I : Normal control (Normal saline-0.1ml)
Group II : Stress control (Negative Control)
Group III : Standard group (*Withania somnifera*/ Ashwagandha) (100 mg/kg)
Group IV: *Orthosiphon stamineus* extract (Aqueous) (200 mg/kg)
Group V : *Orthosiphon stamineus* extract (Aqueous) (400 mg/kg)

2.4.4 Biochemical analysis

The animals were mild anesthetized using ether and the blood samples of each animal were separately collected by retro-orbital puncture and transferred into Eppendroff's tubes (4mL) containing 50 µl of anticoagulant (10% trisodium citrate). Then the plasma was separated and estimated for various biochemical parameters like glucose, cholesterol, triglycerides, BUN, ACTH and blood cell count (RBC, WBC and DLC) [13].

2.4.5 Statistical analysis

All the results were expressed as mean ± standard error of mean (SEM). The statistical significance was determined by using Student's t-test. The significant differences between the groups were determined using a comparison test with the value of $p < 0.05$, $p < 0.01$, $p < 0.001$ which is considered as statistically significant [14,15].

3. RESULTS

3.1 Qualitative Phytochemical Screening

The presence of alkaloids, carbohydrates, glycosides, proteins, amino acids, phenols, triterpenoids, saponins and flavonoids were observed in aqueous extract of *O. stamineus* leaves (Table 1).

Table 1. Qualitative phytochemical screening of *Orthosiphon stamineus* leaves extract

S. no	Test	Aqueous extract
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Glycosides	+
4.	Proteins and amino acids	+
5.	Steroids	-
6.	Fixed oils and fats	-
7.	Phenols	+
8.	Tannins	-
9.	Triterpenoids	+
10.	Saponins	+
11.	Gum and mucilage	-
12.	Flavonoids	+

**(+) = Present; (-) = Absent*

3.2 Effect of Anoxia Stress Tolerance Test on Blood Cell Count

RBC, WBC and DLC count studies were conducted in anoxia stress tolerance in standard (*Withania somnifera*) and aqueous extract of *Orthosiphon stamineus* (200 & 400 mg/kg) treated groups. From the results, it was found that standard (*Withania somnifera*) has significantly increased in RBC, WBC and DLC count. Reduced RBC, WBC and DLC count during stress has been increased with the standard drug, low dose and high dose of aqueous extract. A significant increase was observed in high dose of aqueous extract of *Orthosiphon stamineus* which is almost comparable to standard drug. Results were tabulated in (Table 2).

Student't' test indicates that there was a significant increase in blood cell count in standard and aqueous extract.

3.3 Effect of Anoxia Stress Tolerance Test on Biochemical Parameters

Biochemical parameters like glucose, cholesterol, triglycerides, BUN and ACTH was estimated in anoxia stress tolerance test in rats after treatment with standard (*Withania somnifera*) and aqueous extract (200 & 400 mg/kg) of *Orthosiphon stamineus.* It was found that significant reduction in glucose, cholesterol, triglycerides and BUN level were observed but increases in ACTH estimation level in aqueous extract of *Orthosiphon stamineus*.

Table 2. Effect of aqueous extract of *Orthosiphon stamineus* on blood cell count in Anoxia stress tolerance rats

Group	RBC (ml/L)	WBC (ml/L)	Neutrophils (ml/L)	Lymphocytes (ml/L)	Monocytes (ml/L)	Eosinophils (ml/L)	Basophils (ml/L)
Normal control	9.47±0.03	8.50±0.10	3.50±0.14	4.68±0.09	0.51±0.02	0.20±0.02	0.10±0.02
Stress control	5.59±0.24	3.73±0.03	1.58±0.09	2.23±0.10	0.22±0.01	0.12±0.01	0.05±0.01
Withania somnifera (Standard 100 mg/kg)	8.42±0.09***	7.51±0.29***	2.96±0.10***	3.55±0.38***	0.18±0.02***	0.18±0.01***	0.08±0.01***
Aqueous extract of *O. stamineus* (200 mg/kg)	7.24±0.05***	6.29±0.08***	3.50±0.04***	2.78±0.09***	0.15±0.01***	0.12±0.01***	0.02±0.01***
Aqueous extract of *O. stamineus* (400 mg/kg)	8.20±0.03***	7.06±0.09***	2.38±0.14***	3.01±0.02***	0.15±0.01***	0.15±0.01***	0.05±0.01***

*1. *P < 0.05, **P < 0.01, ***P < 0.001 Withania somnifera VS Stress Control; 2. *P < 0.05, **P < 0.01, ***P < 0.001 extract treated groups VS Stress Control; 3. Values mean ± S.E.M., 4. n=4*

Table 3. Effect of aqueous extract of *Orthosiphon stamineus* on biochemical parameters in Anoxia stress tolerance rats

Group	Glucose (mmol/L)	Cholesterol (mmol/L)	Triglycerides (mmol/L)	BUN (mmo/lL)	ACTH (ng/L)
Normal control	6.88±0.17	1.80±0.06	1.38±0.00	6.19±0.07	467.25±100.75
Stress control	8.89±0.27	2.80±0.02	1.30±0.00	6.80±0.09	294.25±56.75
Withania somnifera (Standard 100 mg/kg)	7.07±0.64***	2.07±0.02*	1.36±0.00	5.62±0.23***	493.5±91.00***
Aqueous extract of *O. stamineus* (200 mg/kg)	8.57±1.00***	1.64±0.05***	1.08±0.02*	6.46±0.09***	340.75±1040.75***
Aqueous extract of *O. stamineus* (400 mg/kg)	7.28±0.06***	1.91±0.11***	1.20±0.07***	6.02±0.15***	381.5±113.00***

*1. *P < 0.05, **P < 0.01, ***P < 0.001 Withania somnifera VS Stress Control; 2. *P < 0.05, **P < 0.01, ***P < 0.001 extract treated groups VS Stress Control; 3. Values mean ± S.E.M., 4. n=4*

Table 4. Effect of aqueous extract of *Orthosiphon stamineus* on blood cell count and biochemical parameters in Noise induced stress rats

Groups	RBC (ml/L)	WBC (ml/L)	Glucose (mmol/L)	Cholesterol (mmol/L)	Triglycerides (mmol/L)	BUN (mmol/L)	ACTH (ng/L)
Normal control	9.30±0.22	8.68±0.16	7.48±0.19	1.83±0.02	1.38±0.00	6.24±0.08	461.75±14.75
Stress control	6.74±0.29	6.48±0.23	10.98±0.27	2.36±0.17	0.30±0.00	7.24±0.46	333.00±21176.00
Withania somnifera (Standard 100 mg/kg)	8.00±0.15***	7.60±1.31***	8.57±0.05***	1.66±0.12***	0.25±0.00***	6.73±0.20***	478.00±712.00*
Aqueous extract of *O. stamineus* (200 mg/kg)	8.04±1.43***	6.70±0.52**	6.12±0.60***	2.21±1.32*	0.66±0.97***	6.40±0.29***	371.25±84.75***
Aqueous extract of *O. stamineus* (400 mg/kg)	8.37±2.45**	7.48±0.50***	7.98±3.42*	1.98±3.19*	1.66±0.43***	6.80±0.05***	483.75±442.75***

*1. *P < 0.05, **P < 0.01, ***P < 0.001 Withania somnifera VS Stress Control; 2. *P < 0.05, **P < 0.01, ***P < 0.001 extract treated groups VS Stress Control; 3. Values mean ± S.E.M., 4. n=4*

Results were tabulated in (Table 3). Student't' test indicates a significant activity with both the extracts.

3.4 Effect of Noise Induced Stress on Blood Cell Count

It was found that aqueous extract has been significantly increased RBC and WBC count which is almost comparable with standard drug. Results were tabulated in (Table 4).

Student't' test indicates there is a significant increase in levels of RBC and WBC count when compared to stress control animals.

3.5 Effect of Noise Induced Stress on Biochemical Parameters

From the study, the aqueous extract exhibited significant activity by reducing, enhanced blood glucose level in high dose of aqueous extract. Whereas there is significant reduction of BUN level and increasing cholesterol triglycerides and ACTH level was observed in high dose of aqueous extract. More effect was observed in high dose of aqueous extract of *Orthosiphon stamineus* than low dose of aqueous extract of *Orthosiphon stamineus*, which is almost comparable with standard drug.

Results were tabulated in (Table 4). Student't' test indicates a significant activity with both the extracts.

4. DISCUSSION

Adaptogen is a term used to describe agents that increase the non- specific resistance of organisms against an assortment of stressors. A recent review is taking place in this field and problems associated with the evaluation of adaptogens. The present study was undertaken to verify the adaptogenic effect of leaf extract of the plant.

Generally, stress will alter various biochemical parameters and blood cell count. Hyperglycaemic response was observed during stress, stress raises serum cholesterol is likely to be related to the enhanced activity of hypothalamo-hypophysial axis resulting increased liberation of catecholamines and corticosteroids. This could lead to increase in blood cholesterol level since; epinephrine is known to mobilize lipids from adipose tissue.

The effect of stress on serum triglycerides has been shown to be variable. It could be suggested that the change in the levels of serum triglycerides is possibly mediated by adrenal medullary secretions and through activation of the sympathetic nervous system.

There will be mobilization of proteins by glucocorticoids leads to enhance urea levels. Due to excessive release of corticosteroids, there is an increase in the blood cell count. In anoxia stress tolerance, there is an increase in anoxia tolerance time with both low and high dose of extracts.

In case of anoxia tolerance and noise induced stress, there is increase in levels of biochemical parameters. The level of increase was more in noise stress compared to anoxia stress tolerance test. There is an increase in the blood cell count in both the stress conditions but in anoxia stress it is more significant when compared to noise stress.

Stress induces adrenomedullary response in rats; adrenaline, in turn, stimulates β_2 receptors on the pituitary gland causing greater release of ACTH. ACTH can stimulate the adrenal medulla and also cortex.

Phytochemical profiles in *Orthosiphon stamineus* indicate the presence of alkaloids, carbohydrates, glycosides, proteins and amino acids, phenols, triterpenoids, saponins and flavoinoids in aqueous extract.

Presence of glycosides, flavonoids, triterpenoids, saponins will confirm the adaptogenic activity as it is compared with the standard drug *Withania somnifera* where it contains glycosides, sterols and flavonoids.

5. CONCLUSION

The result of the present studies clearly indicates a significant adaptogenic (Anti-stress) activity of aqueous extract administered at a dose of 200mg/kg and 400mg/kg in both anoxia stress tolerance and noise induced stress type of experimental models. The constituents of the aqueous leaf extract of *Orthosiphon stamineus* are, alkaloids, carbohydrates, glycosides, proteins and amino acids, phenols, triterpenoids, saponins and flavoinoids.

It is concluded that adaptogenic activity of *Orthosiphon stamineus* (400 mg/kg) is

comparable to the standard *Withania somnifera* (100 mg/kg). The adaptogenic activity of aqueous extract may be due to presence of glycosides, flavoinoids, triterpenoids and saponins those were identified in preliminary phytochemical screening.

The exact mechanism by which *O.stamineus* produces its anti-stress activity cannot be explained with the present data, however, further studies are needed for isolation and characterization of phytochemical constituents of *Orthosiphon stamineus* aqueous leaves extract that may lead to the development of adaptogenic activity that can be used in the control of different types of stress.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee" (Reference No. KPJUC/CRI/BPS/EC/2016/16).

ACKNOWLEDGEMENTS

The authors would like to thank the Management of KPJ Healthcare University College, Kota Seriemas, Nilai, Malaysia, for their continuous encouragement and support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Charles O, Esimone Michael U, Adikwu Chukwuemeka S, Nworu Festus B, Okoye C, Damian C Odimegwu. Adaptogenic potentials of *Camellia sinensis* leaves, *Garcinia kola* and *Kola nitida* seeds. Scientific Research and Essays. 2007;2(7):232–237.
2. Boenisch ED, Haney MC. The stress owner's manual. California: Impact Publishers; 2004.
3. Laxmikant Maruti Purane, Suryadevera Vidyadhara. Antistress activity of *Diospyros Malabarica* (Desr.) Kostel in mice and rats. International Journal of Pharmaceutical Sciences and Research. 2016;7(8):3299-3305.
4. Anonymous. Benefits of misai kuching. Herbs that Promote Health; 2010. Available:http://julea71.blogspot.my/2010/03/orthosiphon-stamineus-or-misai-kucing.html
(Assessed 20 November 2016)
5. Yen CK, Faheem A. A survey of the chemical constituents and biological activities of *Orthosiphon stamineus*. Science International. 2012;24(2):133-138.
6. Manaf A, Harith E, Zaidah R. A glance on medical applications of *Orthosiphon stamineus* and some of its oxidative compounds. Int. J. Pharm. Sci. Rev. Res. 2014;24(2):83-88.
7. Ali R, Marjan H, Jannatul FR, Hasanuzzaman Md, Islam M. Evaluation of thrombolytic potential of three medicinal plants available in Bangladesh, as a potent source of thrombolytic compounds. Avicenna J Phytomed. 2014;4(6):430–436.
8. Anil SB, Kallanagouda RA. Antioxidant and immunomodulatory activity of hydroalcoholic extract and its fractions of leaves of *Ficus benghalensis* Linn. Pharmacognosy Res. 2016;8(1):50–55.
9. Prashant T, Bimlesh K, Mandeep K, Gurpreet K, Harleen K. Phytochemical screening and extraction: A review. Internationale Pharmaceutica Sciencia. 2011;1(1):98-106.
10. Maithili V, Dhanabal SP, Mahendran S, Vadivelan R. Antidiabetic activity of ethanolic extract of tubers of *Dioscorea alata* in alloxan induced diabetic rats. Indian J Pharmacol. 2011;43(4):455-459.
11. Sibi P Ittiyavirah, Sajid KP. Anti stress activity of *Mikania micrantha* Kunth roots in Wistar albino rats. Journal of Scientific and Innovative Research. 2013;2(6):999-1005.
12. Reha Demirel, Hakan Mollaoğlu, Hasan Yeşilyurt, Kağan Üçok, Abdullah Ayçiçek, Muzaffer Akkaya, et al. Noise induces oxidative stress in rat. Eur J Gen Med. 2009;6(1):20-24.
13. Prafulla PA, Pranita PJ, Shirishkumar DA, Bhaskar VH, Tushar S. Adaptogenic activity of lyophilized hydroethanol extract of *Pandanus odoratissimus* in swiss albino mice. International Scholarly Research Notices. 2014;1-10.
14. Alexander NS, Maria AL, Olga NP, Marina NM, Olga VG, Valery GM, et al.

Pharmacological evaluation of *Potentilla alba* L. in mice: Adaptogenic and central nervous system effects. Journal of Pharmaceutical Biology. 2011;49(10): 1023–1028.

15. Shopana Paramasivam, Krupavaram Bethala, AnbuJebaSunilson John Samuel, Anandarajagopal Kalusalingam, Rama Supriya Veeralla. Evaluation of anti-stress activity of *Orthosiphon stamineus* benth. Lamiaceae Methanol Leaves Extract on Wistar Albino Rats, Asian Journal of Research in Medical and Pharmaceutical Sciences. 2017;2(1):1-8.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23752