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A STUDY ON *ANIDRA* WITH SPECIAL REFERENCE TO EFFECT OF *BOERHAVIA DIFFUSA* (*SWETA PUNARNAVA*) ON CERTAIN BIO CHEMICAL ALTERATION INDUCED BY SLEEP DEPRIVATION IN RAT

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KEYWORDS: *Anidra*, Sleep, *Sweta punarnava, B.diffusa.*

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ABSTRACT

Anidra (disturb sleep) remain a significant health problem in the all age group especially in the elder people till date. Free radical reactions have been implicated in the pathology of many diseases. Sleep deprivation has recently been reported to causes oxidative stress and produces free radicals. *B.diffusa* has potent anti oxidant activity. The present study was conducted to assess the effect of *Boerhavia diffusa* (*Sweta punarnava*) decoction on certain bio chemical alteration induced by sleep deprivation in albino rat. The biochemical study revealed that the enzymatic and non enzymatic anti oxidant in *B.biffusa* exhibit preventive role to maintains the cell survival, cellular interaction and also maintain the cell membrane architecture. Oxidative stress plays an important role in the pathophysiology of sleep deprivation, and it indicate that the enhancement of oxidative biomarker (Total Glutathione concentration, M.D.A in terms of lipid per oxidation) indicate that chronic sleep deprivation would actually contribute to oxidative stress, but the present study concluded that scientific evaluation of this plant on disturbed sleep is unclear and it has definite role to check oxidative stress.

INTRODUCTION

Anidra (disturb sleep) is one of the well-known and wide spread health problem. Between 8 to 15 percent of adult population have frequently and chronic complain about the quality and the amount of their sleep.50% of older individual complain disturbed or light sleep, frequent awakening, early morning awakening, undesired day time sleepiness, frequent interruption of sleep by long periods of wakefulness, It is possibly an age dependent intrinsic lightening of sleep homoeostatic process or may be sensitive to environmental stimuli. At least some sleep disturbance seen in older adult, apparently independent of medical or psychiatric illness or often referred as 'age related sleep changes'. Men but not women tend to poorer sleep with ageing. [1]

Causes of *Anidra* includes few Physical factors like Elimination of *Doshas* from the body and head through purgation and emesis, physical exercise bloodletting, fasting, smoke, uncomfortable bed, over work, old age diseases, specially these due to the vitiation of *Vata dosha* and Psychological factor like – Anxiety, anger, fear, predominance of *Sattva* quality and suppression of *Tamas* quality are known to cause of *Anidra*. Some are suffer from *Anidra* even by nature[2]. Loss of sleep is caused by aggravation of *Anil* (*Vata*) and *Pitta*, exhaustions of the mind, loss of tissues and injury to the body.[3] The plant *Boerhavia diffusa* has been traditionally used for the treatment of edema, anasarca gonorrhea, anemia, jaundice, expectorant, laxative etc.[4] and also insomnia (*Nidranash*).[5] Modern research shows that anti oxidant activity and genoprotective action of this plant.[6] The present work has been therefore, undertaken to evaluate the efficacy of *Punarnava* (*B.diffusa*) on sleep deprivation induced changes certain biochemical parameters in animal.

MATERIAL & METHODS

The experimental study was conducted in the laboratory of the Institute of post graduate Ayurvedic Education and Research at SVSP Hospital, 294/3/1,A.P.C Road Kolkata-9, Department of Sharir Samhita. The material and methods used in present study were approved by the IAEC (Institute of Animal ethical Committee).

Animal selection

The animals were collected from a registered breeding institute IICB Kolkata. Albino rats of Sprague Dawley strain of either sex weighing about 200-225 gm. were used in the present experiments. They were kept

under standard housing conditions in a temperature of 24 +-1º C, with a day-night cycle of 12-12 hour. The rats were supplied to standard food and drinking water ad libitum. Experiments were conducted in between 9 A.M to 11A.M.

Selection of Plant Material

The whole plant was collected and properly identified by the Apothecary department of I.P.G.A.E. & R at S.V.S.P. Hospital. Decoction of the whole plant of *Sweta Punarnava* (*B.diffusa* linn) was prepared as per the method described in classical Ayurvedic literature. For preparing decoction, one part of drug is boiled in four parts of water and allows reducing one fourth of the total amount of water. [7]

Induction of Sleep Deprivation

Rats for sleep disruption were placed inside a cage on a grid suspended over water, method developed by Shinomiya et al, with slide modification.[8] In this method, animals were placed on a grid floor (29 x 15 x 7 cm) inside the plastic cage filled with water to 1 cm below the grid surface for 48 hours. The stainless steel rods of the grid (3mm wide) were set 2 cm. apart from each other. Food and water were provided ad libitum.

Experimental Design

The rats were divided into four groups having six rats in each group. The first groups were treated as normal control group (without sleep deprivation) administered equal volume of distilled water. Second group were treated as control group (Sleep deprivation) and administered equal volume of distilled water. Third group were treated with (diazepam and sleep deprivation) as standard group and administered diazepam 2 mg/kg. Body wt. and fourth group (Sleep deprivation and *Punarnava* decoction) and administered decoction of *Punarnova* 5 ml/kg. All the experimental drugs were administered per orally once a day.[9]

Statistical Analysis

All the values were expressed as mean ± SE. The data were analyzed by Students t test followed by analysis of variance (ANOVA). P <0.05 was considered as significant.

OBSERVATIONS AND RESULTS

Effect of sleep deprivation on serum M.D.A concentration (µg/ml of blood) in sleep deprived Rats

48 hours sleep deprivation significantly increases lipid per oxidation in terms of MDA (p<0.01) in control group (Group B; with sleep deprivation) in rats as compared to naive animal (without sleep deprivation).

Effect of Diazepam (2mg\kg bwt) on serum M. D A concentration (µg/ml of blood) in sleep deprived Rats

Pre treatment with Diazepam (2mg\kg bwt) significantly reduces lipid per oxidation in terms of MDA (p<0.01) in Group C (sleep deprivation, treated with Diazepam) in rats as compared to control animal (Group B; sleep deprivation).

Effect of *B.diffusa* decoction (5ml\kg bwt) on serum M. D A concentration (µg/ml of blood) in sleep deprived Rats

Pre treatment with *B.diffusa* decoction (5ml\kg bwt) significantly reduces lipid per oxidation in terms of MDA (p<0.001) in Group D (sleep deprivation, treated with *B.diffusa* decoction) in rats as compared to control animal (Group B; sleep deprivation).

Effect of sleep deprivation on blood Glutathione concentration (µg/ml of blood) in sleep deprived Rats

48 hours sleep deprivation significantly reduces total blood Glutathione concentration (p<0.001) in control animal (Group B; with sleep deprivation) in rats as compared to naive animal (without sleep deprivation).

Effect of Diazepam (2mg\kg bwt) on blood Glutathione concentration (µg/ml of blood) in sleep deprived Rats

Pre treatment with Diazepam (2mg\kg bwt) significantly reversely increases Glutathione concentration (p<0.05) in Group C (sleep deprivation, treated with Diazepam) in rats as compared to control animal (Group B; sleep deprivation).

Effect of *B.diffusa* decoction (5ml\kg bwt) on blood Glutathione concentration (µg/ml of blood) in sleep deprived Rats

Pre treatment with *B.diffusa* decoction (5ml\kg bwt) significantly reversely increases Glutathione concentration (p<0.01) in Group D (sleep deprivation, treated with *B.diffusa* decoction) in rats as compared to control animal (Group B; sleep deprivation).

Effect of sleep deprivation on Blood Glucose level (mg/dl of blood) in sleep deprived Rats

48 hours sleep deprivation significantly increases blood glucose level (p<0.01) in control group (Group B; with sleep deprivation) in rats as compared to naive animal (without sleep deprivation).

Effect of Diazepam (2mg\kg bwt) on Blood Glucose level (mg/dl of blood) in sleep deprived Rats

Pre treatment with Diazepam (2mg\kg bwt) significantly reduces blood glucose level (p>0.05) in Group C (sleep deprivation, treated with Diazepam) in rats as compared to control animal (Group B; sleep deprivation).

Effect of *B.diffusa* decoction (5ml\kg bwt) on blood Glucose level (mg/dl of blood) in sleep deprived Rats

Pre treatment with *B.diffusa* decoction (5ml\kg bwt) significantly reduces blood glucose concentration (p<0.05) in Group D (sleep deprivation, treated with *B.diffusa* decoction) in rats as compared to control animal (Group B; sleep deprivation).

Effect of sleep deprivation on body weight (gms) changes (before and after treatment) of different group in sleep deprived Rats

In Group A, (without sleep deprivation) did not show any significant changes of body weight in comparison between before and after treatment.

In control animal (Group B; with sleep deprivation); 48 hours sleep deprivation significantly reduces body weight in comparison between before and after treatment.

In Group C, (sleep deprivation; treated with Diazepam (2mg\ kg bwt) did not show any significant changes of body weight in comparison between before and after treatment.

In Group D, (sleep deprivation; treated with *B.diffusa* decoction (5ml\ kg bwt) did not shows any significant effect of body weight in comparison between before and after treatment.

Table 1: Effect of *B.diffusa* (decoction) on body weight (gms) changes of different group in sleep deprived Rats

Name of group (n=6)	Before Test (Mean±SEM)	After Test (Mean±SEM)
Group A	134.16±4.72	132.50±3.81*
Group B	138.33±3.33	130.00±3.14**
Group C	140.83±3.27	141.66±2.47*
Group D	135.83±3.00	135.00±3.41*

Group A-control (without sleep deprivation), Group B-experimental control (with sleep deprivation), Group C - sleep deprivation - treated with diazepam (2mg/kg bwt)

Group D- sleep deprivation-treated with *B.diffusa* decoction (5ml/kg bwt), Data were expressed as Mean ±SEM (Stander error of Mean), P<0.05 was consider as significant.*Indicate-P>0.05 (not significant),** Indicate P<0.01.

Table2: Effect of *B.diffusa* (decoction) on different biochemical parameters in sleep deprived Rats

Name of group	M.D.A (Mean±SEM)	Glutathione (Mean±SEM)	Glucose (Mean±SEM)
Group A	3.73±0.19	73.87±1.54	95.65±2.73
Group B	4.51±0.13 [a]	63.59±1.17 [a]	108.87±2.81 [a]
Group C	4.03±0.05 [c]	68.68±1.56 [c]	102.42±2.21 [c]
Group D	3.60±0.15 [d]	71.50±1.88 [d]	99.42±1.84 [d]
One way ANOVA	F=7.76 df=(20,3) p<0.001	F=8.62 df=(20,3) p<0.001	F=5.33 df=(20,3) p<0.001

P<0.05 was consider as significant, Degree of freedom (df)=(20,3), F=F ratio, P [a] <0.01 as compare to Group A.,P [c] <0.05 as compare to Group C., P [d] <0.01 as compare to Group D.

DISCUSSION

Sleep is thought to have powerful restoratives properties. Sleep deprivation in human is widely believe to impact in health system, and is a well known risk factor for development of many diseases. Increase generation of oxidative free radicals or impaired anti oxidant defense mechanism have been implicated in chronic sleep deprivation induced perturbed homeostasis including Immunosuppression, Diabetes mellitus, Acid peptic disorder, Atherosclerosis, Cognitive dysfunction[10] etc.

48 hours sleep deprivation increased lipid per oxidation accompanied by a decreased in glutathione activity is suggesting oxidative stress in sleep deprived animal. Reimund theorized that sleep increases the efficiency of anti oxidant mechanism of the brain.[11]

The serum glucose level was significantly elevated (p<0.01) in sleep deprived control animal, as compared with naïve animal (without sleep deprivation). Elevation of serum glucose concentration in sleep deprived control animal could be due to high level of serum cortisol level. It is well known factor that sleep deprivation is the cause of sympathetic stimulation, which can stimulates adrenocortical hormone secretion like cortisol [12] Disturbed sleep alter the metabolism of glucose with insulin resistant pattern, and cortisol induced insulin resistant, neoglucogenesis that causes increase concentration of serum glucose.[13]

Pre treatment with *B.diffusa* decoction significantly reversely increase (p<0.01) the blood glucose level as compared to control animal (Sleep deprivation). It is very consistence with the other studies. Bhatia V, Kinja K, et al; reported that *B.diffusa* and ethanolic extract exhibits significant anti hyperglycaemic activity in Alloxan induced as well as steptozotocin induced hyperglycaemia in rat. [14]

48 hours Sleep deprivation increased lipid per oxidation (p<0.01) in terms of M.D.A in control animal (Group B) in comparison to naïve animal (Group A). Pre treatment with Diazepam (2mg \kg bwt) significantly reduced serum M.D.A concentration (p<0.001). Serum lipid per oxidation in terms of M.D.A concentration

significantly reduce ($p<0.01$) in group D, treated with decoction of *B.diffusa* (5ml\kg bwt).

The present result indicates that the total Glutathione concentration markedly depleted in sleep deprived rats (Group B) as compared to naïve animal (Group A, without sleep deprivation) and subsequently the glutathione activities reversely accelerated in Group C treated with Diazepam (2mg \kg bwt) ($p<0.05$) and in Group D ($p<0.01$) treated with *B.diffusa* decoction.

Following 5 days of sleep deprivation there was significantly body weight changes found in sleep deprived rats in comparison between before treatment and after treatment in Group B (sleep deprivation) ($p<0.01$). Whereas no potential changes of body weight were found in the treatment groups, both Group C and Group D ($p>0.05$).

Szymusiak R and Satinoff E et al, reported that Experimental animals completely deprived of REM sleep for long period shows lose weight in spite of increase calorie intake[15]. Exposure to sleep deprivation had a long lasting impact was on tissue weight. There was a decrease hemi lateral epididymal fat in mature animal. other study suggested that chronic sleep disturbance leads to weight gain due to low level of Leptin hormone and high level of Ghrelin hormone which stimulates hunger. Other scientific studies also showed that marked reduction in the body weight occur, due to hyper metabolic condition in sleep deprived animal. Marked depletion of blood Glutathione concentration in sleep deprived rat was found in Group B (in sleep deprived rat), which might be due to excessive utilization of Glutathione as an anti oxidant in dependent anti oxidant mechanism. Everson neil hogg et al, reported that metabolic and immunological consequence of sleep deprivation point to a high potential for anti oxidant imbalance. Glutathione is consider a major free radical scavenger that reflex the degree to which a tissue has been oxidative challenge[16]. SA.Comhair, MT.Lewis et al, suggested that Glutathione depiction could result from in appropriate low synthesis rate or from degradation and disposal in sleep deprived rat.[17]

48 hours sleep deprivation increases lipid per oxidation was found in sleep deprived animal in our present study. Recent biochemical reports further demonstrated that lipid per oxidation would severely interrupt the basic physical properties and structural organization of the cell membrane which is believed to be critical involved in several neuropathological and metabolic disturbance.[18]

48 hours sleep deprivation caused significant changes in oxidative parameters and Diazepam was not so much potential anti oxidant activity as compared to *B.diffusa* decoction in sleep deprived animal. Benzodiazepines are widely used drugs for the management of disturbed sleep and associated problem in spite of not having anti oxidant property.

The observe anti oxidant activities of *B.diffusa* may be due to the presence of flavonoid and phenolic compounds. They are known to posses strong anti oxidant properties. Gopal. T.K et al, reported that root of *B.diffusa* were found to reveal anti oxidant potency which support the use of this plant in traditional medicine [19]. Photochemical studies have revealed the presence of alkaloids, flavonoids, phenols, and saponin in *B.diffusa*.[20]

Premkumar P and priya J.et al reported that the extract of *B.diffusa* posses significant level of enzymatic and non enzymatic anti oxidant.[21] The result of enzymatic and non enzymatic anti oxidant in *B. biffusa* exhibit that they possess preventive and productive role to maintains the cell survival cellular interaction and also maintain the cell membrane architecture. *B.diffusa* (*Punarnava*) has been used in varieties of clinical *condition* for a long period but scientific evaluation of this plant on disturbed sleep is unclear.

CONCLUSION

The result of our present study strongly suggested that oxidative stress plays an important role in the patho-physiology of sleep deprivation, and it indicate that the enhancement of oxidative biomarker (Total Glutathione concentration, M.D.A in terms of lipid per oxidation) indicate that chronic sleep deprivation would actually contribute to oxidative stress.

The drug *B.diffusa* (*Punarnava*) has been used in varieties of clinical condition in Ayurveda for a long period but scientific evaluation of this plant on sleep deprivation in experimental Rats is limited. Present experimental study concluded that the *B.diffusa* has protective action against sleep deprivation induced biochemical alteration and it can be consider in the management of sleep deprivation.

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